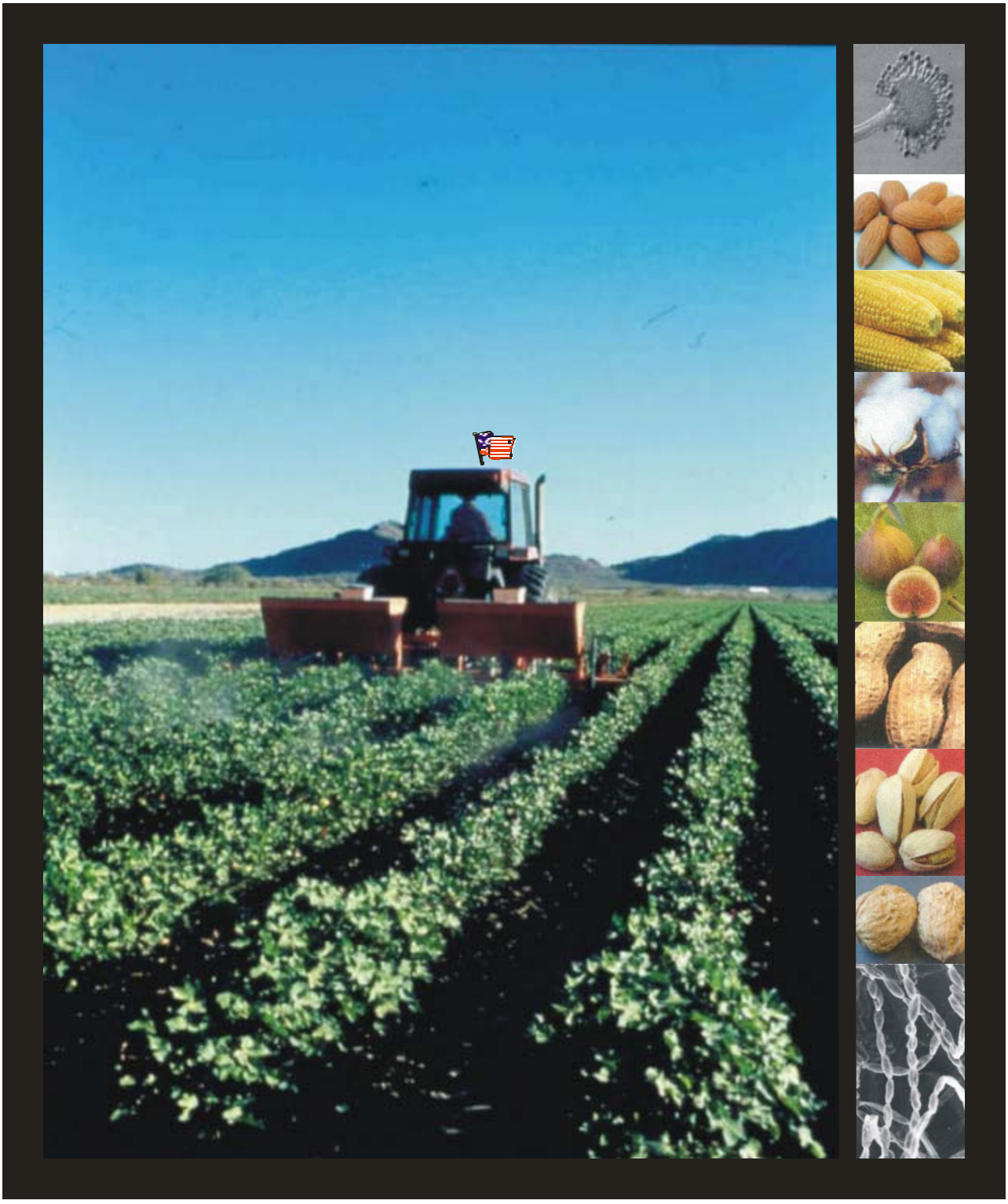


Proceedings of the 1st Fungal Genomics, 2na Fumonisin Elimination and 14th Aflatoxin Elimination Workshops
October 23-26, 2001
Phoenix, Arizona

We thank the cotton industry for sponsoring these workshops!

The Cotton Foundation

Cotton Incorporated

National Cotton Council

National Cottonseed Products Association

Arizona Cotton Research & Protection Council

Proceedings of the 1st Fungal Genomics, 2nd Fumonisin Elimination and 14th Aflatoxin Elimination Workshops, October 23-26 2001, Phoenix, Arizona

CONTENTS

POSTER PRESENTATIONS

POSTER PRESENTATIONS

POSTER PRESENTATIONS

AFLATOXIN/FUMONISIN ELIMINATION AND FUNGAL GENOMICS WORKSHOPS 2001

PHOENIX, ARIZONA
OCTOBER 23-26

Aflatoxin B_1 and fumonisin B_1 are now considered to be the most important food borne mycotoxins worldwide, and the year 2001 was an especially important one from an international regulatory perspective. For example, in addition to the publication of the Food and Drug Administration's Final Guidance for Industry, the Codex Committee on Food Additives requested the 56th Joint Expert Committee on Food Additives (56th JECFA) to evaluate the health risks from fumonisin B_1, B_2 and B_3 in foods. As a result of the evaluation, the 56th JECFA proposed a maximum tolerable daily intake of FB_1, FB_2 and FB_3 of 2 : g/kg of body weight per day. The recommendation will have negligible impact on US corn exports since in most developed countries, consumption of fumonisins fall well below the proposed maximum tolerable daily intake. Nonetheless, the fumonisin risk assessment is a good example of why the US cannot rely simply on sound science to protect US export commodities from unreasonable regulation. For example, if the 56th JECFA had used a safety factor of 1000 rather than 100, then many people in Western Europe and the USA would exceed the proposed maximum tolerable daily intake and the impact on corn exports would be immense. In addition, because the European Commissions reliance on the "precautionary principle" is a driving force in policy decisions, developing and implementing approaches for reducing aflatoxin and fumonisin levels in export commodities is urgently needed if the US is to remain competitive in these markets. Attaining low levels of aflatoxin contamination in export crops is increasingly urgent given the recent EU regulation of 2 ppb aflatoxin B1 (4 ppb total aflatoxins) in nuts, dried fruit, and cereals (see Otsuki et al., 2001, *Food Policy*, **26**:495-514).

Fungal Genomics Workshop. Scientific progress occurs most swiftly through the development of new paths of inquiry. Today that new path of inquiry is genomics, the sequencing, mapping, annotation, and analysis of all the genetic information in a species. The promise of genomics is that it will provide the fundamental knowledge required to understand the complex interaction between the plant host and the fungus and the regulatory circuits that govern mycotoxin biosynthesis. This is not to say that genomics will supplant other avenues of inquiry, such as conventional breeding and hypothesis driven research. The workshop participants were agreed that a concerted effort should be made to obtain the complete genome sequence of *Aspergillus flavus*, Fusarium graminearum and F. verticillioides and that in host plants every effort should be made to identify and develop the means to move resistance genes into important crops.

2nd Fumonisin Elimination Workshop. Fumonisins produced by *Fusarium verticillioides* are primarily a problem in corn and they can co-occur with aflatoxin. While both mycotoxins are liver carcinogens in rodents, only aflatoxin is classified by the International Agency for Research on Cancer as a human carcinogen (Group 1). In 2002, this Agency will re-evaluate both fumonisin (currently Group 2B) and aflatoxin. The fact that they co-occur on corn adds considerable impetus within the US to develop the means to eliminate both mycotoxins. Understanding the factors that control two mycotoxins

occurring simultaneously and developing corn genotypes resistant to both mycotoxins will be extremely difficult, particularly since different genetic factors are likely to control resistance to *Aspergillus* and *Fusarium* species. In addition, Fusarium verticillioides can infect without any indication of disease. The research reported includes field trials with transgenic Bt. corn which have shown reductions in fumonisin levels in the US, France and other countries. The search for corn hybrids with resistance to Fusarium ear rot and fumonisin contamination has identified several commercial lines with clear resistance. Studies are ongoing to develop a better understanding of the genes responsible for kernel resistance to fungal infection and subsequent toxin production. Management strategies such as early harvest and reduction of insect pressure could reduce occurrence of both mycotoxins. Screening using high speed sorting systems that detect infected kernels could reduce contamination in the final products. However, high levels of fumonisin can be found in non-diseased corn. Thus, screening for ear rot or damaged kernels may not reduce fumonisin levels.

14th Aflatoxin Elimination Workshop. Unlike fumonisin, which in the US is only a problem in corn, aflatoxin contaminates many crops. These include corn, peanuts, figs, cottonseed, almonds, walnuts, pistachios and other high oil content crops.

The first defense against aflatoxin contamination of crops is good management practices, including insect control. However, in spite of good management practices, aflatoxin persists as a perennially serious problem in the Southern US and is an episodic problem in the Midwestern US. To develop additional protection, studies are underway using biocompetitive exclusion and conventional breeding to reduce infection with toxigenic strains of *A. flavus* in figs and pistachio nuts. The biochemical mechanisms by which plant constituents increase resistance to insect damage are being studied in corn and other crops. Identification of insect resistance genes will allow selection for resistance traits and replacement in plants where these traits have been lost. Computer programs are being developed to predict when and where fungal infection will appear in corn. Nonetheless, growers must carefully weigh the desire for high yield and reduced cost with the possibility of high level aflatoxin contamination.

Fungi are resilient organisms. The goal of complete elimination of aflatoxin will be difficult to attain. Understanding the physiological and environmental factors that control the interactions between fungi and between the fungus and its host is critical. This has led to successful use of competitive exclusion to control preharvest aflatoxin contamination of cottonseed in Arizona. In 2001, nearly 20,000 acres of Arizona cotton fields were treated with the atoxigenic biocontrol strain AF36. The level of aflatoxin in cottonseed was reduced by 80% compared to untreated fields. This approach is also proving successful for cotton in South Texas, and is now being explored for corn and peanuts and has promise for pistachio nuts and figs. The use of other biocontrol agents such as saprophytic yeast is also being shown to be effective against colonization by toxigenic fungi in almond and pistachio.

Breeding for resistance has the most promise for long-term, effective control. However, in addition to lower levels of aflatoxin, the variety must have agronomic traits that are attractive to the grower. Considerable success has been achieved in developing acceptable almond varieties with resistance to both insect damage and aflatoxin contamination. Similar success is being achieved in identifying resistant peanut genotypes with favorable agronomic characteristics. Success in corn has been more difficult since multiple mechanisms of resistance are necessary to achieve reduced levels of aflatoxin contamination. There is an ongoing concerted effort to identify resistant germplasm, mechanisms of resistance, and molecular markers for resistance that can facilitate development of corn varieties with desirable agronomic traits. Identification of the natural sources of resistance in commercially acceptable varieties is a critical first step to the development of resistant varieties acceptable to growers. Progress has been made in identifying natural constituents of walnut, endogenous enzymes and other proteins in maize, plant growth regulators, and volatile hydrocarbons emitted by pistachio leaves that act as aflatoxin resistance factors, inhibit toxin biosynthesis, or have antifungal properties. These sources of resistance will be useful for developing molecular markers to be used in conventional breeding and with transgenic approaches for reducing aflatoxin in crops.

Identification of the factors controlling toxin production and fungal infection, and a better understanding of the molecular basis for plant resistance are crucial first steps towards development of transgenic varieties resistant to aflatoxin contamination. Crop resistance to aflatoxin through genetic engineering remains largely in the development phase. Genes have been identified that produce proteins that are toxic to invading fungi, however, the expression of these peptides in plants is difficult to monitor and often lack efficacy at target sites. As with conventional breeding approaches, development of transgenic varieties will require introduction of multiple mechanisms of resistance to achieve reduced levels of aflatoxin contamination.

The sponsors of all three 2001 workshops in Phoenix were the National Cotton Council, the National Cottonseed Products Association, the Cotton Foundation, Cotton Incorporated, and the Arizona Cotton Research and Protection Council. USDA-ARS sponsored research to resolve the problem of aflatoxin contamination of cottonseed has met with many successes. This year's workshop featured a site visit to the Arizona Cotton Research and Protection Council Facility in Phoenix. This facility is undoubtedly the best example of what can be accomplished through well funded collaborative research efforts between ARS, industry and university scientists.

DR. JANE ROBENS
National Program Leader
Food Safety and Health
Beltsville Agricultural Research Center
Agricultural Research Service, USDA
Beltsville, MD

DR. RONALD T. RILEY
Research Pharmacologist
Toxicology and Mycotoxin Research Unit
Russell Research Center
Agricultural Research Service, USDA
Athens, GA

AGENDA

2001 AFLATOXIN / FUMONISIN ELIMINATION

AND

FUNGAL GENOMICS WORKSHOPS

OCTOBER 23 – 26, 2001

EMBASSY SUITES – PARADISE VALLEY, PHOENIX, AZ

FUNGAL GENOMICS WORKSHOP

TUESDAY, 23 OCTOBER 2001

RESEARCH STRATEGIES FOR ELIMINATING MYCOTOXINS FROM FOOD IN THE 21ST CENTURY

8:00 10:55 AM **REGISTRATION**

10:55 AM **ANNOUNCEMENTS**

11:00 AM **INTRODUCTORY REMARKS:** Jane Robens, National Program Leader, Food Safety and Health, USDA, ARS, Beltsville, MD.

SESSION 1: **FUNGAL GENOMICS – MOLECULAR APPROACHES FOR ELIMINATING MYCOTOXINS FROM FOODS**

Session Chair – Joan W. Bennett, Tulane University, New Orleans, LA

11:05 AM "Genomics for Filamentous Fungi". Joan W. Bennett. Tulane University, New Orleans, LA.

Overview - Genomics -- the sequencing, mapping, annotation, and analysis of all the genetic information in a species -- has accelerated the pace of gene discovery and become an essential part of the biology of the 21st century. Comparative analysis of filamentous

genomic data makes genetic models, such as *Aspergillus nidulans*, accessible to scientists studying economically important species such as *Aspergillus flavus*, and will facilitate dynamic analysis of what triggers genetic expression of toxin pathways, plant pathogenesis, and other aspects of mold invasion of agricultural commodities.

11:30 AM "*Aspergillus flavus* EST Technology and Its Applications for Eliminating Aflatoxin Contamination." Jiujiang Yu[1], Deepak Bhatnagar[1], Thomas E. Cleveland[1] and William C. Nierman[2]. [1] USDA, ARS, Southern Regional Research Center, New Orleans, LA; [2]The Institute for Genomic Research, Rockville, MD.

Overview - The Expressed Sequence Tag (EST) program at SRRC will be presented. The complete set of genes that are responsible for, or related to, toxin production will be studied. This information will provide vital clues for devising strategies for eliminating aflatoxin contamination of crops.

11:50 AM "Differential Gene Expression on a Genomic Scale: a Tool to Limit Fumonisin Production by *Fusarium verticillioides* and Commodity Losses." Daren W. Brown[1], Robert H. Proctor[1], Anne E. Desjardins[1], Ronald D. Plattner[1] and Catherine Ronning[2]. [1]USDA, ARS Mycotoxin Research, National Center for Agricultural Utilization Research, Peoria, IL; [2] The Institute for Genomic Research, Rockville, MD.

Overview - A program to sequence cDNAs from *F. verticillioides* will be presented. This program will result in the identification of unique fungal genes involved in the corn infection process and production of fumonisins *in planta*. Knowledge from this research will lead to the development of strategies to minimize or eliminate fumonisins from corn.

12:10 PM "Genomics of the Mycotoxin Producing Fungus, *Fusarium graminearum*." H. Corby Kistler. USDA, ARS, Cereal Disease Laboratory and Department of Plant Pathology, University of Minnesota, St. Paul, MN.

Overview - Different strategies may be taken to reduce mycotoxin contamination of crops. These include interference with fungal reproduction, disruption of infection processes and modulation of mycotoxin accumulation in the plant. Each strategy can be approached using genomics to identify mechanisms to be targeted for control. Results of over 10,000 expressed sequence tags (ESTs) will be presented from relevant fungal growth and developmental

stages and during plant infection that hold promise for novel approaches to toxin control.

12:30 – 1:30 PM **WORKING LUNCH**

Discussion Leader

Deepak Bhatnagar, USDA, ARS, Southern Regional Research Center, New Orleans, LA.

Discussion Topic

"Applying the Genomic Wrench – New Tool for an Old Problem"

1:30 PM "Divergence in AflR Regulation Among Aflatoxin-Producing Fungi". Kenneth C. Ehrlich, Beverly G. Montalbano, and Peter J. Cotty. USDA, ARS, Southern Regional Research Center, New Orleans, LA.

Overview - Variability in the aflatoxin biosynthetic pathway regulatory gene among *Aspergillus* species suggests that evolutionarily, aflatoxin production is sensitive to selective pressure from developmental, nutritional and environmental factors. SNPs were identified that may be useful in development of rapid methods for sorting species, strains and isolates.

1:50 PM "Understanding Fumonisin Biosynthesis Through Functional Genomics Studies in *Fusarium verticillioides*". Charles Woloshuk. Botany and Plant Pathology, Purdue University, West Lafayette, IN.

Overview - Using a mutant of *F. verticillioides*, we have determined that corn kernel pH is important in the regulation of fumonisin biosynthesis. DNA sequencing of expressed sequence tag (EST) EST libraries identified several genes that could be involved in fumonisin biosynthesis. The goal is to decipher the cues within the corn kernel that influence fumonisin production using functional genomics techniques to determine the role of putative regulatory genes in fumonisin biosynthesis.

2:10 PM "Gene Expression Profiling in *Aspergillus flavus*". Gary A. Payne. Department of Plant Pathology, North Carolina State University, Raleigh, NC.

Overview - We are using gene expression profiling to identify genes that are differentially expressed during aflatoxin production. Over 600 unique clones have been identified and their expressed sequence

tags (ESTs) have been printed on glass slides. We are currently engaged in profiling the expression of these genes under different conditions known to influence aflatoxin biosynthesis such as carbon and nitrogen source, temperature, and pH.

2:30 PM "The Use of Proteomics to Elucidate Factors Regulating the Corn-*Aspergillus flavus* Interaction". Zhiyuan Chen[1], Robert L. Brown[2], Thomas E. Cleveland[2], Kenneth E. Damann[1]. [1]Department of Plant Pathology and Crop Physiology, Louisiana State University, Baton Rouge, LA; [2]USDA, ARS, Southern Regional Research Center, New Orleans, LA.

Overview - Aflatoxin resistance-associated proteins were identified in resistant maize genotypes. Several proteins, either unique to or upregulated in these lines, when compared to susceptible genotypes, were identified and sequenced. Their possible functions in host resistance against *Aspergillus flavus* infection/aflatoxin contamination are discussed.

2:50 PM "Genomic and Bioinformatic Approaches for Enhancing Strategies for Improving Resistance to *Aspergillus* Ear Rot and Aflatoxin Production in Maize". Torbert Rocheford. Department of Crop Sciences, University of Illinois, Urbana, IL.

Overview - Molecular marker mapping of chromosome segments for resistance to *Aspergillus* Ear Rot and aflatoxin production in maize will be presented in the context of how this mapping information can be related to bioinformatic databases, and how genomic resources in these databases can present candidate genes for molecular marker assisted selection and transgenic efforts.

3:10 - 3:40 PM **BREAK**

3:40 PM **PANEL DISCUSSION**
Panel Chair: Gary A. Payne, Department of Plant Pathology, North Carolina State University, Raleigh, NC.

4:30 – 7:00 PM **DINNER**

SESSION 2: **UNDERSTANDING THE CHALLENGE OF FUNGAL GENOMICS NEW HOPE FOR AN OLD PROBLEM**

Chair - Thomas E. Cleveland, USDA, ARS, Southern Regional Research Center, New Orleans, LA

7:00 PM **INTRODUCTORY REMARKS**

7:05 PM "Mycotoxin Control, What Can Genomics Provide?" William C. Nierman. Vice President for Research, The Institute for Genomic Research, Rockville, MD.

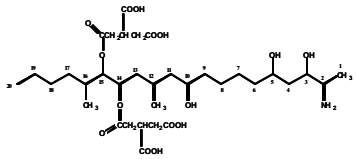
Overview - Genomics provides a description of the entire gene content of an organism. Genomic approaches exemplified by expressed sequence tag (EST) analysis, genome sequencing, and microarray expression analysis reveals the presence and expression pattern of virulence and toxigenic trait related genes in fungi and resistance genes in crop plants. This information will provide a fertile resource for hypothesis driven research to eliminate the detrimental effects of these fungi in vulnerable crops.

7:35 PM "The Power of Genomics: Increasing Value from the Lab, to the Field, and Beyond". Kellye A. Eversole. Eversole Associates, Chevy Chase, MD.

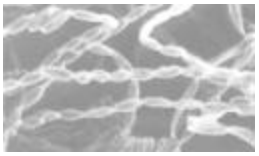
Overview - Genomics has given us the ability to determine the exact location of all genes in an organism and the function of genes, gene clusters, and non-genic regions. What are the benefits of having this information? Why should we care about genomics and genomic sequencing of agricultural crops and related pathogens? What does this mean to growers, livestock producers, industry, consumers, and society in general? These questions and others will be addressed in a general discussion of bringing the power of genomics to bear in agriculture and the resulting benefits of increasing value from the lab to the field and beyond.

8:05 – 8:30 PM **DISCUSSION**

2001 AFLATOXIN / FUMONISIN ELIMINATION WORKSHOPS



2nd FUMONISIN ELIMINATION WORKSHOP



WEDNESDAY, 24 OCTOBER 2001

7:00 AM **REGISTRATION / POSTER ASSIGNMENT**

8:00 AM **ANNOUNCEMENTS**

8:10 AM **WELCOME AND OPENING REMARKS**: Phillip Wakelyn[1] and Larry Antilla[2], [1]National Cotton Council, Washington, DC and [2]Arizona Cotton Research and Protection Council Phoenix, AZ.

SESSION 1: **FUMONISIN CONTAMINATION OF CORN AND DEVELOPMENT OF CELLULAR, BIOLOGICAL, AND ENVIRONMENTAL CONTROL STRATEGIES**

Moderator: Jennifer Snyder, Corn Refiners Association, Inc.

8:20 AM "Reduction of Fumonisin Levels in the Grain of Bt Maize". Bruce Hammond[1], K. Campbell[1], C. Pilcher[1], T. Degooyer[1], A. Robinson[1], D. Melcion[2], B. Cahagnier[2], A. Pietri[3] and G. Piva[3]. [1]Monsanto Company, St. Louis, MO; [2]INRA, Laboratory of Microbiology and Cereal Technology, Nantes, France; [3] School of Agriculture, U.C.S.C., Piacenza, Italy.

8:40 AM "Infection and Fumonisin Production by *Fusarium verticillioides* During Seed Development". Gary Payne, Brian Bush, Marty Carson, Winston Hagler and Marc Cubeta. Department of Plant Pathology, North Carolina State University, Raleigh, NC.

9:00 AM "Fusarium Ear Rot and Fumonisin Contamination: An Indiana Survey 1991-2001". Charles Woloshuk. Botany and Plant Pathology, Purdue University, West Lafayette, IN.

9:20 AM "*Fusarium verticillioides* Conidiation and the Impact on Infection and Disease of Corn Seedlings". Anthony E. Glenn, Dorothy M. Hinton, and Charles W. Bacon. USDA, ARS, Toxicology & Mycotoxin Research Unit, Russell Research Center, Athens, GA.

9:40 – 10:10 AM **BREAK**

10:10 AM "Resistance to Fusarium Ear Rot and Fumonisin Production". Don G. White, Michael J. Clements and Craig E. Kleinschmidt. Department of Crop Science, University of Illinois, Urbana, IL.

10:30 AM "High Speed Sorting Tests for Removing Single Corn Kernels with Aflatoxin and Fumonisin from Asymptomatic Kernels". Floyd E. Dowell[1], Tom Pearson[1] and Don Wicklow[2]. [1]USDA, ARS, Engineering Research Unit, Manhattan, KS; [2]USDA, ARS, Mycotoxin Research Unit, National Center for Agricultural Utilization Research, Peoria, IL.

10:50 AM "A Tissue Specific Expression Cassette for Increasing Fungal Resistance and Reducing Mycotoxin Levels in Maize". Michael J. Muhitch. USDA, ARS, Mycotoxin Research Unit, National Center for Agricultural Utilization Research, Peoria, IL.

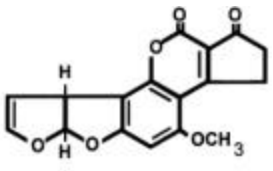
11:10 AM "Organization and Distribution of Fumonisin Biosynthetic Genes in *Fusarium*". Robert Proctor, Daren Brown, Ronald Plattner, and Anne Desjardins. USDA, ARS, Mycotoxin Research Unit, National Center for Agricultural Utilization Research, Peoria, IL.

11:30 – 12:00 AM **PANEL DISCUSSION**
Panel Chair: Tony Glenn. USDA, ARS, Toxicology and Mycotoxin Research Unit, Russell Research Center, Athens, GA.

12:00 – 12:45 PM **BREAK**

12:45 – 5:00 PM **BOX LUNCH AND TOURS OF THE ARIZONA COTTON RESEARCH AND PROTECTION COUNCIL FACILITY AND USDA/CDFA STERILE PINK BOLLWORM MOTH REARING FACILITY**

5:00 – 7:30 PM **OPENING RECEPTION/POSTER VIEWING**



14TH ANNUAL AFLATOXIN ELIMINATION WORKSHOP

THURSDAY, 25 OCTOBER 2001

7:00 AM **REGISTRATION / POSTER ASSIGNMENT**

8:00 AM **ANNOUNCMENTS**

8:05 AM **WELCOME -** Ron Rayner[1] and Chuck Youngker[2]. [1]A Tumbling T Ranches, Goodyear, AZ; [2] Youngker and Youngker Farms, Buckeye, AZ.

SESSION 2: **CROP MANAGEMENT AND HANDLING, INSECT CONTROL AND FUNGAL RELATIONSHIPS**

Moderator: David Ramos, Walnut Marketing Board

8:15 AM "Subsample Preparation of Pistachios". T. F. Schatzki and N. Toyofuku . USDA, ARS, Western Regional Research Center, Albany CA.

8:30 AM "Aflatoxin Control in Pistachios: Removal of Contaminated Nuts, Ecological Relationships, and Biocontrol". Themis Michailides and Mark Doster. University of California, Davis/Kearney Agricultural Center, Davis, CA.

8:45 AM "Aflatoxin Control in Figs: Development of Resistant Cultivars, Identification of Contaminated Fruit and Biocontrol". Mark Doster and Themis Michailides. University of California, Davis/Kearney Agricultural Center, Davis, CA.

9:00 AM "A Functional Approach Towards More Effective Deployment of Multigenic Pest Resistance". Patrick F. Dowd. USDA, ARS, Mycotoxin Research Unit, NCAUR, Peoria, IL.

9:15 AM "Corn Aflatoxin Management System (CAMS): Assessing Risks and a Timeline for Completion". Neil Widstrom[1], Marshall Lamb[2] and Judith Johnson[2]. [1]USDA, ARS, Crop Genetics and Breeding Research Unit, Tifton, GA; [2]USDA, ARS, National Peanut Research Laboratory, Dawson, GA.

9:30 – 9:50 AM **PANEL DISCUSSION**
Panel Chair: Themis Michailides. University of California, Davis/Kearney Agricultural Center, Davis, CA.

9:50 – 10:15 AM **BREAK**

SESSION 3: **MICROBIAL ECOLOGY**

Moderator: Phil Wakelyn, National Cotton Council

10:15 AM "Evaluation of Intraspecific Competition (*Aspergillus flavus* Link) and Aflatoxin Formation Using a Replacement Series". Donald T. Wicklow. USDA, ARS, Mycotoxin Research Unit, National Center for Agricultural Utilization Research, Peoria, IL.

10:30 AM "Role of Competition and Adverse Culture Conditions in Preventing the Loss of Aflatoxin Production by *Aspergillus flavus* During Serial Transfers". Bruce W. Horn and Joe W. Dorner. USDA, ARS, National Peanut Research Laboratory, Dawson, GA.

10:45 AM "Biological Control of Aflatoxin Contamination of Peanuts with Nontoxigenic Strains of *Aspergillus flavus* and *A. parasiticus*". Joe W. Dorner. USDA, ARS, National Peanut Research Laboratory, Dawson, GA.

11:00 AM "Aflatoxin Contamination and Aflatoxin Producing Fungi in South Texas: Initial Experience". Peter J. Cotty. USDA, ARS, Southern Regional Research Center, New Orleans, LA.

11:15 AM "The USDA/ARS - ACRPC Partnership in Aflatoxin Control in Arizona Cotton: Current Status". Larry Antilla[1] and Peter J. Cotty[2]. [1]Arizona Cotton Research and Protection Council, Phoenix, AZ; [2]USDA, ARS, Southern Regional Research Center, New Orleans, LA.

11:30 AM "Biological Control of Aflatoxin in Almonds and Pistachios by Preharvest Yeast Applications". Sui-Sheng T. Hua. USDA, ARS, Western Regional Research Center, Albany, CA.

11:45 – 12:15 PM **PANEL DISCUSSION**
Panel Chair: Donald T. Wicklow. USDA, ARS, Mycotoxin Research Unit, National Center for Agricultural Utilization Research, Peoria, IL.

12:15 – 1:15 AM **LUNCH**

1:15 – 1:45 PM KEYNOTE ADDRESS:
"MYCOTOXIN REGULATORY ISSUES: UNITED STATES STRATEGIES"
Douglas Park, Ph.D.
Director, Division of Natural Products, CFSAN, USFDA, Washington, DC.

SESSION 4: **CROP RESISTANCE – CONVENTIONAL BREEDING**
Moderator: Bob Sacher, Con Agra Grocery Products

1:45 PM "The Identification of Maize Kernel Resistance Traits Through Comparative Evaluation of Aflatoxin-Resistant with -Susceptible Germplasm". Robert L. Brown[1], Thomas E. Cleveland[1], Zhiyuan Chen[2], Shirley V. Gembeh[1], Abebe Menkir[3], Steve Moore[4], Daniel Jeffers[5], Kenneth E. Damann[2], and Deepak Bhatnagar[1]. [1]USDA, ARS, Southern Regional Research Center, New Orleans, LA; [2]Department of Plant Pathology and Crop Physiology, Louisiana State University, Baton Rouge, LA; [3]International Institute of Tropical Agriculture, Ibadan, Nigeria;[4]Dean Lee Research Station, Louisiana State University, Alexandria, LA; [5]International Maize and Wheat Improvement Center, El Batan, Mexico.

2:00 PM "Molecular Genetic Analysis of Resistance Mechanisms to Aflatoxin formation in Corn and Peanut". Baozhu Z. Guo, [1]Neal W. Widstrom,[2] C. Corley Holbrook,[2] R. D. Lee,[3] and R. E. Lynch[1]. [1]USDA, ARS, Crop Protection and Management Research Unit and [2]Crop Genetics and Breeding Research Unit, Tifton, GA; [3] University of Georgia, Coastal Plain Experiment Station, Tifton, GA.

2:15 PM "Breeding and Molecular Marker Assisted Selection for Resistance to *Aspergillus* Ear Rot and Aflatoxin Production". Don White and Torbert Rocheford, Department of Crop Sciences, University of Illinois, Urbana, IL.

2:30 PM "Selection of Peanut Breeding Lines with Drought Tolerance and Resistance to Preharvest Aflatoxin Contamination". C. Corley Holbrook[1], Dave M. Wilson[2], Diane L. Rowland[3], C. K. Kvien, and Jeff P. Wilson[1]. [1]USDA, ARS, Crop Genetics and Breeding, Tifton, GA; [2]Plant Pathology, University of Georgia, Athens, GA; USDA, ARS, [3]Peanut Research Laboratory, Dawson, GA.

2:45-3:15 PM **BREAK**

3:15 PM "Maize Germplasm Evaluation for Aflatoxin Resistance in Texas". Javier Betrán[1], Tom Isakeit[2], Gary Odvody[2], and Kerry Mayfield[1]. [1] Soil & Crop Sciences Department, Texas A&M University, College Station, TX; [2]Pathology Department, Texas A&M University, College Station, TX.

3:30 PM "Field Performance of Seed and Endocarp Based Resistance to Preharvest Aflatoxin Contamination In Almond". Thomas M. Gradziel and Abhaya Dandekar. Department of Pomology, University of California, Davis, CA.

3:45 – 4:15 PM **PANEL DISCUSSION**
Panel Chair: Don White. Department of Crop Sciences, University of Illinois, Urbana, IL.

4:15 – 5:00 PM **POSTER VIEWING**

5:00 PM **COMMODITY BREAKOUT SESSIONS**

7:00 PM **BANQUET**

FRIDAY, 26 OCTOBER 2001

8:30 AM **ANNOUNCMENTS**

SESSION 5: **CROP RESISTANCE – GENETIC ENGINEERING**
Moderator: Larry Antilla. Arizona Cotton Research and Protection Council

8:45 AM "Development of Transgenic Peanuts with Enhanced Resistance Against Preharvest Aflatoxin Contamination". Arthur K. Weissinger. Crop Science, North Carolina State University, Raleigh, NC.

9:00 AM "Transgenic Peanut for Preharvest Aflatoxin Reduction." Peggy Ozias-Akins[1], Hongyu Yang[1], Yoko Akasaka[1], Chen Niu[1] and Robert Lynch[2]. [1]Department of Horticulture, University of Georgia, Tifton, GA; [2]USDA, ARS, Coastal Plain Experiment Station, Tifton, GA.

9:15 AM "Antimicrobial Peptide Technology to Prevent Fungal and Bacterial Diseases of Crops". Paul Zorner. Demegen Inc., Pittsburgh, PA.

9:30 AM "Expression Cassettes to Confer Resistance to *Aspergillus flavus* in Cotton". Caryl A. Chlan. University of Louisiana at Lafayette, LA.

9:45 AM "Antimicrobial Assays with Transgenic Cottons". Kanniah Rajasekaran, J.W. Cary, T.J. Jacks and Thomas E. Cleveland. USDA, ARS, Southern Regional Research Center, New Orleans, LA.

10:00 – 10:30 AM BREAK

10:30 AM "Progress Toward Determining if Alpha-Amylase Inhibitors Can Reduce Aflatoxin Contamination in Maize". Charles Woloshuk. Botany and Plant Pathology, Purdue University, West Lafayette, IN.

10:45 AM "Genetic Engineering and Breeding of Walnuts for Control of Aflatoxin". Abhaya M. Dandekar[1], Gale McGranahan[1], Patrick Vail[2], Russell Molyneux[3], Noreen Mahoney[3], Charles Leslie[1], Sandie Uratsu[1], Ryann Muir[1] and Steven Tebbets[2]. [1] Department of Pomology, University of California, Davis CA; [2]Horticultural Crops Research Laboratory, Fresno CA; [3]UDSA/ARS Western Regional Research Center, Albany, CA.

11:00 – 11:30 AM PANEL DISCUSSION
Panel Chair: Peggy Ozias-Akins. Department of Horticulture, University of Georgia, Tifton, GA.

11:30 – 12:30 PM LUNCH

SESSION 6: **POTENTIAL USE OF NATURAL PRODUCTS FOR PREVENTION OF FUNGAL INVASION AND / OR AFLATOXIN BIOSYNTHESIS IN CROPS**

Moderator: Bob Klein, California Pistachio Commission

12:30 PM "Anti-Aflatoxigenic Constituents of 'Tulare' Walnut". Russell J. Molyneux[1], Noreen Mahoney[1], Bruce C. Campbell[1], Gale McGranahan[2] and Jim McKenna[2]. [1]USDA, ARS, Western Regional Research Center, Albany, CA; [2]Department of Pomology, University of California, Davis, CA.

12:45 PM "Influence of Naphthalene-Acetic Acid on Growth, Differentiation and Mycotoxin Synthesis by *Aspergillus nidulans* and *A. parasiticus*". Carlos M. Bucio-Villalobos[1],Hugo A. Luna[2], and Doralinda Guzman-de-Pena[3] . [1]Instituto de Ciencias Agricolas, Universidad de Guanajuato, Mexico; [2]Facultad de Ciencias Biologicas, Universidad Autonoma de Nuevo Leon, Mexico; [3]Laboratorio de Micotoxinas, Unidad-Irapuato. Centro de Investigacion y Estudios Avanzados IPN, Mexico.

1:00 PM "Characterization of a Maize Kernel Protein Associated with Resistance Against *Aspergillus flavus* Infection/Aflatoxin Production". Z. -Y. Chen[1], R.L. Brown[2], K.E. Damann[1], T. E. Cleveland[2]. [1]Department of Plant Pathology and Crop Physiology, Louisiana State University Agricultural Center, Baton Rouge, LA; [2]Southern Regional Research Center, USDA, ARS, New Orleans, LA.

1:15 PM "Volatiles from Leaves and Fruit of Pistachio Species". Gloria B. Merrill and James N. Roitman. Plant Mycotoxin Research Group, USDA, Western Regional Research Center, Albany, CA.

1:30 PM "Genetic Analysis of Inhibitory Proteins from Maize Seeds". Gary A. Payne and Rebecca S. Boston. Departments of Plant Pathology and Botany. North Carolina State University. Raleigh, NC.

1:45 PM "Using GFP *Aspergillus flavus* Strains to Assess Aflatoxin Resistance of Peanut Under Drought". Keith Ingram[1], Corley Holbrook[2], Arthur Wiessinger[3], Dave Wilson[4]. [1]University of Georgia, Crop and Soil Sciences, Griffin, GA; [2]USDA, ARS, Tifton, GA; [3]Crop Science, North Carolina State University, Raleigh, NC; [4] Plant Pathology, University of Georgia, Tifton, GA.

2:00 PM "*Aspergillus* EST Databases Provide Insights into Aflatoxin Biosynthesis and Its Regulation". Perng-Kuang Chang[1], Jiujiang Yu[1], Deepak Bhatnagar[1], Peter Cotty[1], Nancy Keller[2], Rodolfo Aramayo[3], Bruce Roe[4], Doris Kupfer[4], *Aspergillus oryzae* EST Consortium[5], and Thomas Cleveland[1]. [1]Southern Regional Research Center, ARS-USDA, New Orleans, LA; [2]University of Wisconsin, Madison, WI; [3]Texas A&M University, College Station, TX; [4]University of Oklahoma, Norman, OK. [5]National Institute of Advanced Industrial Science and Technology, Ibaraki, Japan.

2:!5 – 2:45 PM **PANEL DISCUSSION**
Panel Chair: Russell J. Molyneux. USDA, ARS, Western Regional Research Center, Albany, CA.

2:45 – 3:00 PM **CLOSING REMARKS** Jane Robens. National Program Leader Food Safety and Health, USDA, ARS, Beltsville, MD.

FUNGAL GENOMICS WORKSHOP

SESSION 1: FUNGAL GENOMICS – MOLECULAR APPROACHES FOR ELIMINATING MYCOTOXINS FROM FOODS

Session Chair – *Joan W. Bennett, Tulane University, New Orleans, LA*

GENOMICS FOR FILAMENTOUS FUNGI

J. W. Bennett. Department of Cell and Molecular Biology. Tulane University, New Orleans, LA

Genomics is the new paradigm for 21st century biology. Defined as the analysis of all the genetic endowment in a species, genomics has transformed biology into an information science. The tools of genomic analyses include the use of large databases (e.g. GenBank) that are accessible to the public via the Internet; high speed automated DNA sequencing; and intensive computer analysis of these data. The volume of new DNA submissions is staggering and the ability to analyze DNA sequence data lags far behind our ability to accumulate them. In the annotation phase of genomic analysis of a newly sequenced species, computational tools such as Genefinder, Artemis, and Grail are used to predict open reading frames and other motifs in sequenced DNA. One extracted, predicted genes are analyzed for homologies to known genes from other species. During annotation, proteins are automatically placed into predefined functional or structural categories. Genomics research has created a need for higher order analyses. If the genome is the analysis of the DNA sequence, then the "transcriptome" is the analysis of all expressed genes (the complete set of messenger RNAs); the "proteome" is all the proteins (the complete set of proteins), and the "metabolome" is the complete set of metabolites.

The first organisms sequenced were the bacteria *Haemophilus influenzae* and *Mycoplasma genitalium* in work done by The Institute for Genetics Research (TIGR) and published in 1995. However, it was the European Union that took the lead in fungal genomics by funding most of the collaborative effort that led to the completion of the genome sequence of the yeast *Saccharomyces cerevisiae* in 1996. Shortly thereafter, the First International Conference on Fungal Genomics was held at Oklahoma State University, Stillwater, Oklahoma, where it was determined that a pilot project on chromosome IV of *Aspergillus nidulans* be instituted. A subsequent workshop held in New Orleans in December 1996 added *Neurospora crassa* to the target list of filamentous fungi. Subsequently, a number of medically and agriculturally important fungi became the subject of genomics projects including: *Ashbya gossypii, Candida albicans, Cryptococcus neoformans, Histoplasma capsulatum, Magnaporthe grisea, Phytophthora infestans*, and *Pneumocystis carinii*. The genus *Aspergillus* has also received attention with projects for *A. fumigatus, A. niger, A. oryzae* and A. *nidulans* in various stages of completion. In addition, a robust Expressed Sequence Tag (ETS) project is underway for the aflatoxin producer, *A. flavus*. During 2001, draft sequences of the first two filamentous fungal species were completed: *Neurospora crassa* and *Phanerochaete chrysosporium*.

Promises of genomic research on fungi include a better understanding of mycotoxin pathways, of virulence factors in both plant and animal pathogens, and the uncovering of new targets for use in screening for antifungal compounds. Comparative analysis of filamentous fungal genomic data will inform the agricultural research of the 21st century and become an essential tool in the battle against crop losses due to fungi.

Aspergillus flavus EST TECHNOLOGY AND ITS APPLICATIONS FOR ELIMINATING AFLATOXIN CONTAMINATION

Jiujiang Yu[1], Deepak Bhatnagar[1], Thomas E. Cleveland[1] and William C. Nierman[2]. [1]USDA/ARS, Southern Regional Research Center, New Orleans, LA and [2]The Institute for Genomic Research, Rockville, MD

Acquisition of genomic sequence information is an innovative strategy for rapidly identifying genes and understanding their functions in a genome. The *Aspergillus flavus* Expressed Sequence Tag (EST) program at this lab (SRRC) is aimed at understanding the genetic control and regulation of toxin production by potential regulator(s) upstream of *aflR*, the mechanism of toxin production in response to internal and external factors, the relationship between primary and secondary metabolism, plant-fungal interaction and fungal pathogenicity as well as evolutionary biology. A normalized cDNA library is being made currently and will be sequenced at The Institute for Genomic Research (TIGR). The sequence information will be used for this study and made available to the public as soon as it is available. *A. flavus* EST technology will identify the majority, if not all, of the genes expressed in fungal genome. A microarray, made from the EST sequences, will be used to detect a whole set of genes expressed under specific environmental conditions. This technology will allow us to study a complete set of fungal genes, simultaneously that are responsible for or related to toxin production. In close cooperation with Dr. Abe and Dr. Gomi, Tohoku university, Japan and Dr. Machida, National Institute of Biosciences and Human Technology, Japan, significant progress has been made in the *A. oryzae* EST program in Japan. *A. oryzae* is a close relative of *A. flavus* except that the industrial strain *A. oryzae* lost the ability to produce aflatoxins. We have full access to their EST data and materials. A 2,000 element gene array has been made available to us at the cost price. An important application of EST and microarray technology is solving the problem of aflatoxin contamination of crops. The *A. flavus* EST program in SRRC is expected to provide valuable information on turning on and off aflatoxin production in the fungal system. This information will provide vital clues for identifying anti-fungal gene(s) or aflatoxin-inhibitory gene(s). These gene(s) could be introduced into commercial crops such as corn and cotton for eliminating aflatoxin contamination of pre-harvest crops.

DIFFERENTIAL GENE EXPRESSION ON A GENOMIC SCALE: A TOOL TO LIMIT FUMONISIN PRODUCTION BY *Fusarium verticillioides* AND COMMODITY LOSSES

Daren W. Brown, Robert H. Proctor, Anne E. Desjardins, and Ronald D. Plattner. Mycotoxin Research, National Center for Agricultural Utilization Research, ARS, Peoria, IL

The filamentous fungus *Fusarium verticillioides* is one of the most prevalent pathogens of maize worldwide. In most cases, the fungus is innocuous: it grows endophytically and can be detected in most maize in the U.S. But, under the right conditions, the fungus can turn pathogenic, causing significant ear or stalk rot. In addition, the fungus can make fumonisins, a family of mycotoxins that, after ingestion, can inflict serious toxicological effects to a variety of farm animals. Moreover, there is an epidemiological correlation between human esophageal cancer and the consumption of fumonisin-contaminated maize in regions of China and South Africa. The best strategy to keep fumonisins from entering the food supply is to prevent them from being produced in the first place. A significant portion of the Mycotoxin Research Units effort at the Agricultural Research Service National Center for Agricultural Utilization Research (ARS-NCAUR) under the USDA National Program for Food Safety in Peoria, Illinois, is directed toward this task. Our research is focused on understanding, at the genetic and physiological levels, how fumonisin biosynthesis is initiated and regulated by the fungus in maize, as well as how fumonisin biosynthesis and fungal growth are influenced by maize itself.

Patterns of differential gene expression have given investigators valuable leads to understanding biological processes in a variety of experimental systems. We, in collaboration with TIGR (The Institute for Genomic Research), are embarking on a program to sequence cDNAs from *F. verticillioides* that represent genes expressed under varying environmental and nutritional regimes and different growth stages of the fungus. Particular attention will be paid to genes differentially expressed after growth on corn tissue; either corn ears saturated with mycelia and/or disease symptoms or corn tissue cultures. We look forward to identifying unique fungal genes involved in pathogenesis (the corn infection process) and the biosynthesis and regulation of fumonisins *in planta*. After identifying genes of interest, gene disruption followed by fermentation/infection studies will explore their function. Knowledge from this research is expected to lead to the development of strategies to minimize or eliminate fumonisins from corn.

Background-Expressed sequence tags (EST's) are partial sequences of cDNA clones. cDNA libraries derived from RNA isolated from *F. verticillioides* will be prepared after fungal growth on a variety of media and environmental regimes. We hope to develop a database representing up to 70% of all the mRNAs present. A set of high-quality, non-redundant transcripts will be used in the future for more extensive functional annotation as well as gene-discovery and mapping experiments. In particular, we are looking forward to conducting microarray expression analysis to allow mRNA expression to be assessed on a genomic scale: the parallel assessment of gene expression of thousands of genes in a single experiment.

GENOMICS OF THE MYCOTOXIN PRODUCING FUNGUS, *Fusarium graminearum* (*Gibberella zeae*)

Frances Trail[1], Jin-Rong Xu[2], Phillip San Miguel[2] and H. Corby Kistler[3].
[1] Michigan State University, East Lansing, MI; [2] Purdue University, West Lafayette, IN; [3]ARS-USDA, Cereal Disease Laboratory and University of Minnesota, St. Paul MN.

Several different strategies may be taken to reduce mycotoxin contamination of crops. These strategies include interference with fungal reproduction, disruption of infection processes and modulation of mycotoxin accumulation in the plant. Each of these strategies can be approached using genomics to identify key mechanisms that may be targeted for control. *Fusarium graminearum* (sexual state: *Gibberella zeae*) causes head blight (also known as scab) of wheat, barley, and oats, as well as foot and crown rot of corn. The pathogen poses a two-fold threat: first, infested cereals show significant reduction in seed quality and yield due to discolored, shriveled kernels, and secondly, scabby grain is often contaminated with trichothecene and estrogenic mycotoxins, making it unsuitable for food or feed. Recent scab outbreaks in Asia, Canada, Europe, South America and the United States highlight the increased threat this disease poses to food supplies worldwide. A genomics approach to the study of *F. graminearum* is critical because for head blight, like many *Fusarium* diseases, effective fungicides and highly resistant plant varieties are not available. A better understanding of the biology of the scab organism is warranted to develop new control strategies. Our long term goals are: (1) to understand the genetic basis for inoculum development, mycotoxin production and pathogenicity; (2) to use genomics to develop a biology-based control program for scab, using the genomics programs of wheat and corn to enhance this project. We have sequenced and analyzed a cohort of over 10,000 ESTs from three cDNA libraries representing different culture conditions and developmental stages of the fungus. From an input of 10,281 EST sequences, 1,200 contigs and 1315 singleton sequences were obtained. Therefore, the estimated number of genes identified by our current EST database is 2515. These efforts are a prelude to the whole genome sequencing of one strain of *F. graminearum*. Genome sequencing not only provides the most complete description of an organism's biological processes and metabolic capability but also provides direct access to the reagents needed for subsequent functional studies. For every organism sequenced to date, research has instantly shifted from local gene-based approaches to more global, genomic approaches. This project will also provide useful information to scientists working with other *Fusarium* species or other fungi for comparative studies of fungal morphogenesis, development and pathogenesis.

APPLYING THE GENOMIC WRENCH - NEW TOOL FOR AN OLD PROBLEM

Deepak Bhatnagar, Jiujiang Yu and Thomas E. Cleveland. USDA, ARS,
Southern Regional Research Center, New Orleans, LA 70124.

Animal disease due to consumption of aflatoxin contaminated feed was first reported in 1964. For the first time in almost 4 decades, it appears that control measures for this problem are within reach. To hasten this process, additional information is needed rather rapidly particularly to understand the specific genetic factors (both in the plant and the fungus) during host-plant fungus interaction. The field of genomics provides us with the best and quickest method to achieve this understanding (as discussed by others in this section, and by the authors in the attached item on *A. flavus* genomics).

The term Agenome@ has existed for over 75 years and refers to an organism=s complete set of chromosomes and the genes contained therein. The term Agenomics@, now universally accepted, was coined recently (in 1986) by Thomas Roderick to describe the scientific discipline of mapping, sequencing and analyzing genomes. As more and more genomes are being sequenced, the emphasis has shifted from just mapping and sequencing to understanding the functions of the genome. Therefore, genome analysis is now being divided into "structural genomics" and "functional genomics". Structural genomics is defined (P. Hieter and M. Boguski, 1997, Science 278:601) as the "initial phase of genomic analysis" with a clear end point that results in the construction of high resolution genetic, physical and transcript maps of an organism. The ultimate physical map of an organism is its complete DNA sequence, whereas, functional genomics is the making use of the vast resource of structural genomics information. Specifically, functional genomics refers to the development and application of global (genome-wide or system-wide) experimental approaches to assess gene function by making use of the information and reagents provided by structural genomics. The fundamental strategy in a functional genomics approach is to expand the scope of biological investigation from studying single genes or proteins to studying all genes or proteins at once in a systematic fashion@. This information is obtained by high throughput methodologies (such as microarray technology) with statistical and computational analysis of results.

Functional genomics is needed to speed up our understanding of the field biology of the fungus in relationship to its interaction with the host plant. But this insight into the behavior of these biological systems first requires the sequence information of the mycotoxigenic fungi of interest namely *Aspergillus flavus* (aflatoxins), *Fusarium verticillioides* (fumonisins) and *F. graminearum* (trichothecenes). These fungal genomes are relatively large and the cost to sequence these are prohibitive. Therefore, funds will have to be raised from a number of sources such as competitive grants (USDA Food Safety NRI grants), industrial support, congressional appropriations and internal funds of the research organizations involved in the project. Within the scientific community, there is significant priority assigned to the fungal species of interest. So, obtaining funds could be accomplished. An educational component (outreach activities) to the project would also enable a favorable review of the proposal by funding sources identified. In addition, significant information is already available on these genomes, and internal funds already allocated to conduct preliminary investigations towards obtaining the complete genome sequences of the fungi of interest

Aspergillus flavus GENOMICS FOR ELIMINATION OFAFLATOXIN CONTAMINATION

Jiujiang Yu, Deepak Bhatnagar and Thomas E. Cleveland. USDA/ARS, Southern Regional Research Center, New Orleans, Louisiana 70124

Aspergillus flavus genomics can be used to study the genetic control and regulation of aflatoxin production, fungal pathogenicity, the plant-microbe interaction, as well as the evolutionary process in aflatoxigenic fungi. The knowledge obtained through *Aspergillus flavus* genomics can benefit farmers in developing strategies to eliminate pre-harvest aflatoxin contamination resulting in a safer, economically viable food and feed supply.

Inhibit aflatoxin formation: *Aspergillus flavus* genomics or EST/Microarray, provides us a tool to identify all of the genes and enzymes in a fungal system involved in or related to aflatoxin production. Some of the critical regulatory components and gene(s) can be targeted through identification of potential genes or enzyme factors from host plant that exhibit an inhibitory effect on aflatoxin formation. These genes or factors can be transferred to commercial crops, corn or cotton, through traditional breeding or genetic engineering to develop anti-aflatoxin crops.

Prevent fungal infection: Through *Aspergillus flavus* genomics, we can have a better understanding of the fungal pathogenicity and mechanism of plant-microbe interaction. The virulence related genes or genes for fungal infection of crops could be identified. This information can be shared with plant breeders to design corresponding breeding objectives to target and neutralize the expression of these virulence genes through genetic engineering.

Inhibit normal fungal growth and development: Aflatoxin formation is based on fungal growth. There are hundreds or even thousands of fungal growth related genes that are vital for fungal survival and propagation. These genes and corresponding enzymes (alpha-amylases, glucosidases, nitrite and nitrate reductases) are critical in a biological cascade reaction. Plant factors that could inhibit fungal growth can be identified and used as fungicides to kill the fungi.

Develop bio-competitive agents for biocontrol: *Aspergillus flavus* genomics can help to better understand genetic diversity and evolutionary biology of the aflatoxigenic and non-toxigenic strains in field isolates. This information could lead to the development of non-toxigenic strains that could be used as bio-competitive agents to displace the toxigenic strains in the field.

Additional benefits: Some atoxigenic *A. flavus* fungi can degrade lignin for paper processing. Some *A. flavus* fungi that produce high amounts of chinases capable of penetrating insect skin (chitin) and colonizing insects, could serve as control agents to replace chemical pesticides. Many useful enzymes have yet to be discovered from *Aspergillus* species for fermentation industry and sugar production (cellulases). Enzymes of high industrial value can be identified using microarray technology.

DIVERGENCE IN *aflR* REGULATION AMONG AFLATOXIN-PRODUCING FUNGI

Kenneth C. Ehrlich, Beverly G. Montalbano and Peter J. Cotty. USDA-ARS, Southern Regional Research Center, New Orleans, LA

Diversity among free-living organisms can result from geographical isolation as well as adaptation to the nutrient supply, environment, microbial competition, and predation. To assess genetic diversity within aflatoxin-producing *Aspergillus* populations, we have compared the sequence of aflatoxin biosynthetic pathway regulatory gene, *aflR*, homologs from *Aspergillus nomius, bombycis, parasiticus, flavus*, and *pseudotamarii* isolates. The comparison showed that there is considerable variability in promoter consensus elements responsive to nitrate, pH and developmental status and in coding region motifs associated with increased proteolytic turnover. Regulatory differences imply that aflatoxin production has evolved to respond to a variety of environmental stimuli and that selective pressures exerted on lineages differed during their divergence. Our results argue against frequent horizontal transfer of the aflatoxin gene cluster, as has been postulated by some, and for evolution of aflatoxin biosynthetic genes at a rate commensurate with that of other non-clustered genes in the fungi. Within a defined *Aspergillus* species genetically distinct isolates are found that are separated by a vegetative compatibility (VCG) system. In order to infer evolutionary relationships among *A. flavus* isolates, to examine gene flow among isolates, to develop non-ambiguous strain identification methods to aid in fungal community monitoring, and to determine the role of aflatoxin and other traits in adaptive success of different VCGs, a study of single nucleotide polymorphisms (SNPs) in different clades and VCGs has been initiated. Based on polymorphisms in the *aflR/aflJ* intergenic region, isolates classified morphologically as *A. flavus* separated into at least seven distinct clades. In some cases individual clades were distinguished by a non-random abundance of transition or transversion mutations. These studies show that adaptive pressures have selected for differential regulation of aflatoxin biosynthesis even within a single species of *Aspergillus* possessing some or all of the genes necessary for aflatoxin production. Therefore, we hypothesize that expression of aflatoxin-pathway genes may be finely regulated in different isolates from the same species.

UNDERSTANDING FUMONISIN BIOSYNTHESIS THROUGH FUNCTIONAL GENOMICS STUDIES IN *Fusarium verticillioides*

Charles Woloshuk, Joseph Flaherty, and Won-Bo Shim. Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN

Recently a mutation in *Fusarium verticillioides* was identified that affected the regulation of fumonisin biosynthesis and conidiation when grown on cracked corn. In the mutant, the polyketide synthase gene for fumonisin biosynthesis, *fum5,* is not expressed when the strain is grown on maize. The mutated gene named *fcc1* encodes a protein similar to cyclin C (UME3) in *S. cerevisiae* (Shim, W-B. and Woloshuk, C. P. 2001. Appl. Environ. Microbiol. 67:1607-1612).

To examine the differences in gene expression between a wild-type and the *fcc1* mutant while growing on corn, forward and reverse subtraction libraries were made consisting of 1500 clones each. Approximately 800 cDNA clones from each subtraction library were sequenced and analyzed via BLASTX. The size of the inserts ranged from 150 bp to 700 bp with an average size of ~350 bp. The wild-type library (forward) has 352 unique ESTs and the mutant library (reverse) has 412 unique ESTs. The two libraries have 49 clones in common. Clones containing inserts with high similarity to known genes (P value $<10^{-5}$) were further categorized into eight groups: carbohydrate metabolism, protein metabolism, fatty acid metabolism, secondary metabolism, cell differentiation, pH response, stress response and signal transduction. The two libraries are contrastingly different. The wild-type library contains three clones in the pH responsive category, while the mutant library contains none. The wild-type subtraction library also contains ten unique genes in the category of fatty acid metabolism compared to one in the mutant library. The mutant library contains ten unique genes in the category of carbon metabolism compared to two genes in the wild-type library. The mutant library also contains nine stress-related genes compared to none in the wild-type library. Genes identified by Proctor et al. (Genbank accession number AF155773) that cluster around *fum5* and are thought to be involved in fumonisin biosynthesis, were identified in the wild-type library but not in the mutant library.

We are currently testing the hypothesis that several genes identified in the wild-type subtraction library are involved in regulating fumonisin biosynthesis. Included are genes that encode a putative cyclin-dependant kinase, DNA binding proteins, and a facilitator of protein-protein interactions. We have isolated cosmids harboring the genes and constructed gene-knockout vectors based on the EST sequences.

GENE EXPRESSION PROFILING IN *Aspergillus flavus*

Gary A. Payne. Department of Plant Pathology, North Carolina State University, Raleigh, NC

Induction of aflatoxin biosynthesis involves the interplay of transcriptional regulatory elements and physiological factors that affect fungal metabolism. Our objective is to identify those genes differentially expressed during aflatoxin accumulation and then profile the expression of the differentially expressed genes during aflatoxin biosynthesis.

Candidate genes for study were identified by hybridizing an array of 100,000 *A. flavus* cDNA clones with probes made from mRNA collected from cultures of the fungus in the log phase of aflatoxin production. A subset of clones expressed primarily during aflatoxin biosynthesis was chosen for sequence analysis. Quality EST sequences (100 base pairs or greater with at least phred 20) were obtained for over 4000 clones. Sequence analysis revealed the presence of over 700 unique clones. Approximately half of the unique EST's represent genes with no known function. Among the EST's with homology to sequences of known functions are those coding for signal transduction pathways, secondary metabolism, glucose regulation, cell wall biosynthesis, and cell cycle control. We have developed a glass slide microarray containing replicates of our unique clone set and we are currently engaged in profiling the expression of these genes under different conditions known to influence aflatoxin biosynthesis such as carbon and nitrogen source, temperature, and pH. Our goal is to identify a common set of expressed genes to be further studied for their function in aflatoxin biosynthesis.

THE USE OF PROTEOMICS TO ELUCIDATE FACTORS REGULATING THE CORN-*Aspergillus flavus* INTERACTION

Zhi-Yuan Chen[1], Robert L. Brown[2], Thomas E. Cleveland[2], Kenneth E. Damann[1]. [1]Dept. Plant Pathology and Crop Physiology, Louisiana State University Ag. Center, Baton Rouge, LA; [2]Southern Regional Research Center, USDA-ARS, New Orleans, LA

Aflatoxins are carcinogens produced by *Aspergillus flavus* and *A. parasiticus* during infection of susceptible crops such as corn, and cottonseed. Though resistant corn genotypes have been identified, the incorporation of resistance into commercial lines has been slow due to the lack of selectable markers. Here we report the identification of potential markers associated with resistance in corn using a proteomics approach. Endosperm and embryo proteins from several resistant and susceptible genotypes have been compared using large format 2-D gel electrophoresis. Comparisons of reproducibly detected spots have found both quantitative and qualitative differences between resistant and susceptible genotypes. Over 20 such protein spots from embryo and endosperm have been sequenced using ESI-MS/MS and Edman degradation after trypsin digestion. Based on peptide sequence homology analysis, these potential markers belong to the following categories: storage proteins (globulin 1 and globulin 2, and late embryogenesis abundant proteins that are related to drought or desiccation, such as LEA3 and LEA14), stress-related proteins (aldose reductase, glyoxalase I, heat shock protein 16.9 and 17.2, peroxiredoxin antioxidant, water stress-inducible protein 18), and antifungal proteins (trypsin inhibitor, pathogenesis-related protein 10). Possession of the unique or of higher levels of these hydrophilic storage proteins, stress-related proteins, and antifungal proteins, may put resistant lines in an advantageous position over susceptible genotypes in the ability to synthesize proteins and defend against pathogens under stress conditions. Therefore, genetic engineering to enhance host resistance may need to include not only antifungal genes, but also stress-related genes.

GENOMIC AND BIOINFORMATIC APPROACHES FOR ENHANCING STRATEGIES FOR IMPROVING RESISTANCE TO *Aspergillus* EAR ROT AND AFLATOXIN PRODUCTION

Torbert Rocheford, Chandra Paul, and Don White. Department of Crop Sciences, University of Illinois, Urbana, IL

In this workshop we heard about extensive fungal resources & tools being developed and used: cDNAs, ESTs, comparative models, RNA profiling, different strains and species. These tools are being aimed at understanding and hopefully eventually disrupting plant pathogenesis and expression of toxin pathways. We also heard about efforts ongoing on the plant side designed to reduce toxin production. For example, corn kernel pH may be important in regulation of fumonisin biosynthesis; carbon, nitrogen, temperature and pH may influence aflatoxin biosynthetic gene expression; aflatoxin resistance-associated proteins have been identified and sequenced in maize; and quantitative trait loci have been associated with resistance to aflatoxins in maize. How do we pull these collective efforts together to achieve higher, more stable levels of resistance in plants? Bioinformatic and genomic tools, approaches, and resources could be used to pull together and summarize the different types of data in a way that may reveal useful interrelationships.

We present examples from our work and then relate these to other research. We and other researchers have mapped quantitative trait loci for resistance to aflatoxin in different genetic backgrounds. We are performing marker-assisted selection and developing near-isogenic lines. However we have only defined chromosome regions and have not identified the underlying genes involved in resistance. The advent of maize cDNAs and microarrays will enable performing RNA profiling on the near-isogenic lines to potentially identify candidate genes involved in resistance. Similarly, RNA profiling can be performed on the fungus in the different near-isogenic lines to identify genes that may be associated with toxin production. Another approach is that known cDNAs can be mapped throughout the maize genome and those that map to the regions of quantitative trait loci (QTL) become candidate genes as well. For example, a chitinase gene has been mapped to the same region as a QTL for resistance. One of the problems with marker assisted selection is that large regions of the chromosome are selected, if we are able to identify genes underlying resistance, then favorable alleles could be selected, reducing linkage drag problems. Identification of genes also provides candidates for transgenic approaches to resistance.

cDNA or genomic DNA sequences of candidate genes provides a common denominator to facilitate bioinformatic comparisons. For example, if proteins potentially associated with resistance are sequenced and converted to DNA sequences, these DNAs could be mapped and related to locations of mapped QTL. As the maize genome is sequenced, then any research that can be converted to a DNA sequence can be used to identify a genome location, and facilitate pooling of diverse sets of research results through databasing and bioinformatic tools into common genome locations. For example, genes that influence kernel composition and thus aflatoxin production could be located and related to other data such as QTL locations. The bioinformatic approaches using cDNAs and/or genomic sequence will enable pooling of information on genes influencing resistance to other fungal pathogens, and may reveal clusters of genes for

resistance. As candidate gene sequences develop, these genes can be sequenced in different lines and associations with resistance potentially made through associative genetics approaches. The bioinformatic databases need to be organized so that information that is published or not published, such as minor QTL associations or a marginal association of a nutrient with aflatoxin production, can be easily accessed in a useful format by all researchers.

FUNGAL GENOMICS WORKSHOP

SESSION 2: UNDERSTANDING THE CHALLENGE OF FUNGAL GENOMICS NEW HOPE FOR AN OLD PROBLEM

Session Chair – *Thomas E. Cleveland, USDA- ARS, Southern Regional Research Center, New Orleans, LA*

MYCOTOXIN CONTROL, WHAT CAN GENOMICS PROVIDE?

William C. Nierman. Vice President for Research, The Institute for Genomic Research, Rockville, MD

Genomics is the relatively new component of biological exploration that focuses on the study of an organism from the perspective of its entire genetic content. This it is not hypothesis based, but discovery based, perhaps reverting to the methodology like the earlier era of observational biology conducted in the manner of Darwin and Wallace. The most well established tools in the genomics enterprise are EST sequencing, genome sequencing, and microarray expression analysis. The kinds of data generated in these projects will be reviewed by illustrations from *Arabidopsis thaliana* so that the potential of genomics approaches to biological problems can be evaluated.

EST Sequencing. EST projects sequence the 5' or 3' or both ends of cDNA clones as a quick, cheap, and easy way to gain a view of the expressed genes of an organism. These **E**xpressed **S**equence **T**ags generate a sequence resource that provides a view of the gene content of and organism as well as tissues and temporal specific data about where and when a gene is expressed. It is typically the first genomics resource developed for an organism with a large genome and for many organisms will be the only resource developed.

If the EST resource becomes sufficiently large, informatics tools such as those applied in building TIGR Gene Indices provide a more extensive resource than a simple listing of EST sequences. This kind of analysis can lead to the development of tentative consensus sequences (TCs), the identification of splice variants, the identification of candidate orthologs across species, and can aid in the annotation of genomic sequence.

Genome Sequencing. Genome sequencing has gained prominence with the human genome project, the completion of the sequence of the genome of an increasing number of other species, and the dramatic decline in the cost of genome sequencing. Genome sequencing provides visibility of all the genes in an organism, provides a view of the evolutionary history of its genome, affords the opportunity to make cross species genome comparisons, and reveals that no information is available for identifying function for 40 – 60% of the genes in an organism. This is true even for well studies organisms such as *Escherichia coli* and *Saccharomyces cerevisiae*. The genome sequence provides a basic resource for nearly all future studies on an organism once the sequence is determined.

Microarray Expression. **.** Analysis: Glass slide microarrays with hybridization targets for each open reading frame (ORF) in a genome constitutes a very powerful tool for determining alterations in the levels of mRNAs in response to altered conditions. The use of appropriate informatics tools allows the identification of transcripts that are coordinately regulated, and to the extent that function can be identified by gene sequence analysis, regulatory components in the response can be identified. Thus, in a single hybridization analysis, most of the genes whose expression levels are altered by an altered condition, can be identified. It is likely that these will be most of the genes that participate in the organisms response to the altered condition.

What Can Genomics Provide? With the completion of the annotated sequence of the genomes of toxigenic fungi, a full description of the genetic networks that control

fungal invasive mechanisms, toxin production, ecosystem survival, and the details of plant-fungus interactions will be within reach. Analysis of global transcription patterns, proteomics of stable and unstable proteins, and genetic and biochemical analysis of phosphotransfer to regulatory proteins, will generate a comprehensive map of the genetic circuitry of these processes. Such an understanding will greatly aid in the development of powerful approaches for mycotoxin control.

THE POWER OF GENOMICS: INCREASING VALUE FROM THE LAB, TO THE FIELD, AND BEYOND

Kellye A. Eversole. Eversole Associates, Chevy Chase, MD.

Despite varying forms and methods of agricultural policy implemented by the U.S. government for the past 70 years or so, there has been a continued, steady decline in the number of growers and ranchers. During this same period, agricultural productivity skyrocketed. Improvements in science and technology, not Federal commodity programs, have been the key to the increased productivity that enabled the growers and ranchers that remained on the farms and ranches to prosper and flourish along with the rest of society. The future of agriculture in the U.S. and abroad will be based on genomics and the accompanying technological advancements that will offer growers and ranchers an opportunity for sustainable profit.

To capture the sustainable profit, we must enable the power of genomics to increase value from the lab, to the field, and beyond. This requires a five-step process of understanding the fundamental genetic information of organisms, identifying customer needs and opportunities, building a bridge with genomics between fundamental science and application, using knowledge derived to create new products and technologies, and establishing new business opportunities for growers and ranchers.

Genomics, the scientific discipline of mapping, sequencing, and analyzing genomes, will give us the fundamental knowledge we need because genomics will allow us to determine the exact location of all genes in an organism and it will facilitate the discovery of the function of genes, gene clusters, and non-genic regions. The power of genomics is in enabling system-wide studies through high throughput technologies – we can view the organism on a grand scale. Before, we were limited to looking at one gene or a few genes and regulatory regions at a time. Today, whole genome sequencing can give us all of the genes and all of the regulatory regions that control how genes are turned on and off, as well as the so-called "junk DNA" the purpose or utility of which is presently unknown. Genomics and protein content (derived through proteomics) will lead to understanding the content, function, and regulation of every gene, genome organization, and biochemical pathway. We will then be able to discover which attributes are inherited and which are acquired and how the interaction between genetics and the environment affect key economic traits, such as susceptibility to diseases and yield. Having the whole genome will shave 2-5 years off the development time for new varieties, hybrids, and traits of agricultural crops.

What are the benefits of having this information? Why should we care about genomics and genomic sequencing of agricultural crops and related pathogens? What does this mean to growers, livestock producers, industry, consumers, and society in general? Agricultural growers suffer as much as $30 billion per year in losses due to plant diseases. Over the past few years, the U.S. has spent more than $200 million in research to eradicate citrus canker, all to no avail. We lack the ability to rapidly identify plant pathogens and this often allows contaminated grain to enter the supply chain unnoticed and spread disease. Genomics can enable the development of rapid diagnostic reagents that can prevent the spread of diseases. Today, hundreds of school children cannot eat peanut butter. Tomorrow, genomics can identify and enable us to turn off the production

of the peanut allergen enabling everyone to enjoy peanuts. Genomics will enable the development of disease and stress resistant crops, it will provide us with the opportunity to turn genes "on and off" or genetically modify or turn off the production of specific proteins. In addition, it will enable us to resist or thwart acts of bioterrorism.

For growers, genomics and the accompanying technologies will accelerate traditional commodity-based improvements, such as enhanced nitrogen efficiency and photosynthesis and drought tolerance, growth rate, and yield. Further, it will provide new opportunities that are not based on the traditional selling of a bulk commodity, such as edible vaccines, therapeutic antibodies, novel proteins, industrial feedstocks tailored for specific end uses, industrial enzymes, and nutritionally enhanced products that will ultimately lead to "nutragenomics".

Genomics is the new paradigm for biological research, as well. It will drive the technological revolution for the rest of this century. To maintain a competitive edge, it is essential to have the "best and brightest" on your team. Yet, they want to work with cutting-edge technologies, not the old, 20th century methods. The best and brightest will choose the systems and funding streams that afford them the opportunity to fulfill their dreams of revolutionary, groundbreaking research. If your organism is not at the leading edge of genomics, you will not get the best and brightest on your side. The science in your crop or other organism will become stagnant. There will be an ever-dwindling number of scientists working on improvements for your crops. Further, without the opportunity for state of the art research that can result in high revenue streams, plant-breeding efforts will decrease significantly over time. The bottom line for growers and ranchers will be reduced profitability.

With structural and functional genomics, an ever-increasing scientific, talent pool will be working to solve problems faced by growers and processors. There will be increased opportunities for developing products and traits that will meet the needs of consumers and manufacturers. The bottom line is that there will be significant opportunities for increased income from increased yields and new crops and there will be an increase in the number of opportunities for growers to own or participate in businesses higher up the value chain.

PANEL DISCUSSION: Fungal Genomics Workshop

Panel Chair: Gary A. Payne

Panel Members: Joan W. Bennett, Deepak Bhatnagar, Daren W. Brown, Zhiyuan Chen, Kenneth C. Ehrlich, Kelly Eversole, H. Corby Kistler, William Nierman, Gary Payne, Torbert Rocheford, Charles Woloshuk, and Jiujiang Yu

Overview and Summary of Discussion: Moderators for the two sessions were Joan Bennett and Ed Cleveland. This was the first year that the Aflatoxin Elimination Workshop has had sessions dedicated solely to fungal genomics. Clearly, mycotoxicologists have a new tool to use in the control and management of mycotoxins. Genomics—the sequencing, mapping, annotation, and analysis of all the genetic information in a species—was shown by presenters in this workshop to be a powerful new tool to better understand gene expression and complex interactions. What problem could be more suited for such a tool than mycotoxin contamination? While we have garnered much information on the epidemiology, ecology, and management of mycotoxigenic fungi, we still know little about the complex interaction between the host and the fungus and the regulatory circuits that governs mycotoxin biosynthesis. Such a lack of information is hindering the development of a comprehensive and stable management strategy for the control of mycotoxins.

The excellent presentations given at this workshop provided an outline of how genomic sciences will facilitate the analysis of what triggers gene expression in pathogenesis and mycotoxin production, and how genomics can be used to study naturally occurring variation within mycotoxigenic species. Several labs are currently generating expressed sequence tags (ESTs) libraries for *Aspergillus. flavus* and *Fusarium graminearum* and *F. verticillioides* and beginning to employ the power of microarray technology to address complex regulatory circuits within these fungi. Information was presented also on plant genomics. The most aggressive public sequencing project for plants is on the model plant *Arabidopsis*. Comparative genomics between *Arabidopsis* and agronomic plants such as corn should aid in our understanding of host resistance. Corn is the subject of a major sequencing project as well, and already a number of ESTs have been catalogued. A major focus of several labs is to map these ESTs in maize to create a finer resolution genetic map. Resistance to mycotoxin producing fungi appears to be quantitative, with each of several genes contributing some degree of resistance. Such resistance is not easily identified or moved into agronomically important genotypes. High throughput marker technology along with the mapping of more ESTs and quantitative traits loci would greatly facilitate the breeding for resistance to mycotoxin producing fungi.

The discussion that followed the presentations focused on ways to facilitate the advancement of a genomics approach to mycotoxin contamination. There was clear agreement that a concerted effort should be mounted to obtain the complete genome sequence of *A. flavus*, *F. graminearum* and *F. verticillioides*. Such information would greatly aid ongoing research in a number of labs that are beginning to generate EST databases for these fungi and to use microarray technology. The availability of the entire genome would allow us to examine the simultaneous expression of all of the fungal genes

during pathogenesis and mycotoxin biosynthesis. We would be able to study complex regulatory circuits associated with mycotoxin production under a number of conditions. Gene expression analysis of the fungus and host plant during infection would also allow us to characterize the cross talk between the host and fungus. Understanding host recognition is likely a key to developing new control strategies.
The group was also in strong support of high throughput mapping as a strategy to identify and move resistance genes into agronomically important genotypes. Such an approach would also facilitate the pyramiding of resistance genes.

A key limitation to genomic sciences is the expense of the research. This is true for sequencing, gene expression analysis, and high throughput mapping. Few granting agencies provide adequate funds for a large-scale genomics project. An adequate and consistent source of funding is needed to support this type of research. There was much discussion about the approach necessary to obtain such funding. Two approaches were deemed the most appropriate. One approach is to try to obtain additional appropriated funds from the USDA to support this research. Such funding support would be justified on the importance of mycotoxins as a threat to food safety and food security in the US and in other countries. A white paper will be drafted and circulated soon justifying the need for more research funding. A second approach is to obtain joint funding from a number of granting agencies. It was suggested that a workshop be held in Washington, DC with a several funding agencies. At this proposed workshop, researchers from our group would outline a comprehensive approach to study mycotoxin contamination.

In conclusion, the group was very supportive of a genomics approach to studying mycotoxins. Genomics and the molecular tools associated with genomic sciences have greatly accelerated the pace of discovery in other areas of science, particularly those associated with human and animal health, and we anticipate that they will have an equal contribution to mycotoxin research. Certainly, as a research community we are prepared to adopt these technologies and put them to use in the many disciplines that are involved in mycotoxin research. This was the first Fungal Genomics Workshop, but it was part of the 14th Aflatoxin Elimination Workshop and the 2nd Fumonisin Elimination Workshop. As a group we have had years of experience working together on this complex problem. Our strength comes from the expertise of scientists from many disciplines that have formed collaborations to address the contamination of food sources by mycotoxins produced by *Aspergillus* and *Fusarium* species.

2nd FUMONISIN ELIMINATION WORKSHOP

SESSION 1: FUMONISIN CONTAMINATION OF CORN AND DEVELOPMENT OF CELLULAR, BIOLOGICAL, AND ENVIRONMENTAL CONTROL STRATEGIES

Moderator: ***Jennifer Snyder, Corn Refiners Association, Inc.***

REDUCTION OF FUNGAL AND FUMONISIN LEVELS IN *Bt.* CORN

B. Hammond[1], K. Campbell[1], C. Pilcher[1], T. Degooyer[1], A. Robinson[1], L. Rice[2], A. Pietri[3], G. Piva[3], D. Melcion[4] and B. Cahagnier[4]. [1]Monsanto Company, St. Louis, MO; [2]USDA-ARS, Ames, IA; [3]School of Agriculture, U.C.S.C., Piacenza, Italy; [4]INRA, Laboratory of Microbiology and Cereal Technology, Nantes, France

Biotechnology has made it possible to develop corn hybrids that are protected against lepidopteron insect pests. Genes that code for *Bacillus thuringiensis* (*Bt.*) Cry proteins have been introduced into corn plants. Cry proteins are the active insecticidal ingredients of *Bt.* microbial insecticides that have been safely used on agricultural crops around the world for 40 years.

Monsanto Company has introduced the Cry1Ab protein in corn plants that protect against lepidopteran insect pests such as the European corn borer (*Ostrinia nubilalis*), fall army worm (*Spodoptera frugiperda*) and the Southwestern corn borer (*Diatraea grandiosella*). Using a CaMV 35S gene promoter, the Cry1Ab protein is produced throughout corn plant tissues (10-15 ppm leaves and stalk, 0.5 ppm grain) providing season-long protection against lepidopteran pests. While season long protection has benefits for the farmer and environment following reduced application of chemical insecticides, there is growing evidence of another potentially important health benefit of *Bt.* protected corn; reduction of fumonisin mycotoxin contamination of corn grain.

Scientists at Iowa State University were the first to report that *Bt.* protected corn had reduced ear rot and fumonisin contamination when compared to near isogenic, non-transgenic controls. This reduction was attributed to reduced insect damage to the corn kernel caused by corn borers decreasing *Fusarium* infection. *Fusarium* species can live in association with corn tissues (endophytes) or they can enter wounded corn tissues damaged by insect feeding leading to infection of the plant. This protection was most evident in varieties (such as Monsanto's event MON 810 corn – YieldGard ®) where the Cry protein was expressed in all plant issues throughout the growing season. Additional field trials with *Bt.* corn have been conducted in the U.S., France, Spain and Italy. All the trials were conducted under conditions of natural insect infestations except for trials conducted at universities in the U.S. where manual infestation with lepidopteran insect pests was used. The analytical procedures used to detect fumonisins in corn grain were based on accepted high performance liquid chromatography methods.

Results of these trials established on average, an 8 fold reduction in fumonisins in *Bt.* corn grown in Italy (1999), a 30 fold reduction in fumonisins in *Bt.* corn grown in France (1997-99) and a 2-3 fold reduction in fumonisin levels in *Bt.* corn grown in the United States (2000). Similar reductions have been reported by others monitoring fumonisin levels in different commercial *Bt.* varieties. Reduction in fumonisin levels could have important health implications for corn grown in countries where mycotoxin contamination is high and corn represents a major portion of the diet. For example, daily dietary exposure to fumonisin in Gunangxi China ranges up to 15.8 ug/kg body weight/day. These farmers consume on average 400 grams of corn per day that is contaminated with aflatoxin and fumonisins; there is an increased incidence of hepatocellular carcinoma in areas of China with high mycotoxin contamination of corn.

If *Bt* corn reduced typical fumonisin contamination by 3 fold, this would reduce daily fumonisin exposure to approximately 5.3 ug/kg/day, which is closer to the 2 ug/kg provisional maximum tolerable daily intake (PMTDI) established by JECFA. To more fully evaluate the potential reduction of fumonisin levels in *Bt*. corn grown in other world areas, additional trials are being initiated with *Bt*.corn grown in South Africa, the Philippines, South America and other countries. New varieties of insect protected *Bt*. corn are also under development that provide protection against a broader spectrum of insect pests and may possibly provide greater reduction in mycotoxin contamination of grain.

INFECTION AND FUMONISIN PRODUCTION BY *Fusarium verticillioides* DURING SEED DEVELOPMENT

Gary Payne, Brian Bush, Marty Carson, Winston Hagler and Marc Cubeta.
Departments of Plant Pathology and Poultry Science, North Carolina State University, Raleigh, NC

Fumonisin contamination of corn grain is a concern for growers because of its toxicity. Currently, no genotypes of corn can be recommended as resistant to fumonisin contamination. Therefore, management strategies are important for the control of fumonisins until plant resistance can be identified. The overall objective of this study was to characterize the temporal profile of seed infection by *Fusarium verticillioides* and fumonisin accumulation in corn kernels to determine if early harvest may be an option for reducing fumonisin contamination. Corn was planted in two locations in North Carolina in 1999 and 2000 and harvested at weekly intervals for 14 weeks after pollination. The percent kernel infection and fumonisin concentrations were determined.

A surprise finding of this study was how soon fumonisin appeared after infection of field grown corn with *F. verticillioides*. A significant number of infected kernels occurred 4-5 weeks after anthesis and fumonisin B1 appeared a week later. An exception to this occurred in Clayton in 2000. An early time of infection at this site preceded fumonisin accumulation by two weeks. In all cases, fumonisin concentrations reached a peak two weeks after its appearance.

From our study it is clear that harvesting early could have the advantage of reducing fumonisin accumulation. The first peak in fumonisin accumulation occurred when kernel moisture was around 20%. In 2000, harvesting of corn with 24% moisture would have resulted in less fumonisin accumulation. The benefits of the early harvest would have to be weighed against the added cost of drying the corn. However, the added costs of drying would be justified in years conducive for fumonisin accumulation.

It is also clear from this study that leaving corn in the field after it reaches 20% moisture is risky in North Carolina. In both years of the study we found a second peak in fumonisin accumulation late in the season. It is not clear what prompted the second increase in infection and fumonisin contamination. It could be associated with late season rains, but there was no clear association of increased kernel infection and fumonisin accumulation with kernel moisture late in the season. Because we found the number of infected kernels and the concentration of fumonisin to be very variable in corn left in the field for an extended period, we suggest that ratings for resistance in a disease screening program be taken during the average harvest date for corn.

Even though the environmental conditions were quite different for the two years, the number of infected kernels was similar across the years. At Plymouth, a maximum of 38% of the kernels were infected in 2000 that was similar to the 42% observed in 1999. Similarly, the maximum percentage of kernels infected of 58% at Clayton for 2000 was similar to the 62% observed in 1999. Fumonisin concentrations, however, were higher in 1999 than in 2000. The data indicate that favorable environmental conditions for fumonisin accumulation may not be the same as those for kernel infection. Such favorable conditions may influence the degree of kernel colonization or may have a direct effect on fumonisin biosynthesis.

Fusarium EAR ROT AND FUMONISIN CONTAMINATION: AN INDIANA SURVEY 1991-2001

Charles P. Woloshuk[1], Ralph Gann[2] and R.J. Everson[3]. [1]Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN; [2]Indiana Agricultural Statistics Service; [3]Animal Disease Diagnostic Laboratory, Purdue University, West Lafayette, IN

In response to a severe drought in 1988 that brought high levels of aflatoxin into the Indiana corn crop, Purdue University launched a program in 1989 to survey Indiana cornfields to determine the level of pre-harvest ear rots and mycotoxins. Each year, the Indiana Agricultural Statistics Service (IASS) randomly selected the fields to be sampled in this study. Corn acres throughout the state had an equal probability of being selected. Two sites in each field are sampled during late August though September prior to harvest. Samples consist of the primary ears from five consecutive plants in a single row. The samples, with the husks left on the ears, were placed in cloth bags and mailed to Purdue University. Upon arriving at Purdue, the ears were husked and examined for symptoms and signs of ear rots. Each year, samples from about 160 fields were examined. Data were recorded for the percentage of kernels visibly infected by each type of the following ear rots: *Fusarium* ear rot, *Gibberella* ear rot, *Aspergillus* ear rot, *Diplodia* ear rot, and minor ear rots caused by *Alternaria*, *Nigrospora*, *Penicillium* and *Trichoderma*. Samples having disease severity of 10 percent or greater were tested for mycotoxins (aflatoxin, ochratoxin, zearalenone, deoxynivalenol and fumonisin).

Fumonisins were first analyzed in 1991. In that year, the incidence and severity of *Fusarium* ear rot were high with 43% of the ears having 3% or more damage per affected ear. Fumonisins were detected in 101 of the 113 samples tested. Fifty samples contained greater that 50 ppm fumonisins. From 1992 to 2001 the incidence and severity of *Fusarium* ear rot has declined. Levels higher than 1 ppm have not been detected since 1995 and in the past two years no samples warranted mycotoxin analysis. The decrease in *Fusarium* ear rot is apparently not part of general decrease in all ear rot diseases. In 2000, *Diplodia* ear rot was a major disease throughout Indiana.

Fusarium verticillioides CONIDIATION AND THE IMPACT ON INFECTION AND DISEASE OF CORN SEEDLINGS

Anthony E. Glenn, Dorothy M. Hinton and Charles W. Bacon. USDA-ARS, Russell Research Center, Toxicology & Mycotoxin Research Unit, Athens, GA

Endophytic infection of corn by *Fusarium verticillioides* can occur at several stages of development throughout the life of the plant. Systemic infections of vegetative and reproductive tissues or more targeted infections via ear silks can both result in infection of developing corn kernels (1, 2). Such infections are of major agronomic interest due to development of kernel rot or ear rot, which potentially leads to increased contamination of corn-based products with mycotoxins such as the fumonisins. In terms of host-pathogen interactions, the ultimate result of kernel infection is that the fungus is well positioned to maternally propagate itself via seed transmission and infect the new seedling that develops from the planted kernel. Interactions at the seedling stage are of interest for identifying cellular and genetic mechanisms responsible for fungal infection and endophytic colonization, as well as fungal physiological adaptations that allow or enhance infection and endophytic growth.The cyclic hydroxamic acids DIMBOA and DIBOA are preformed antimicrobial compounds synthesized to high concentrations during the early days of corn seedling development. These highly reactive compounds ($t_{1/2}$ = 24 h) spontaneously degrade to the corresponding benzoxazolinones, MBOA and BOA, which are also antimicrobial. Prior work from our research unit demonstrated that *F. verticillioides* is tolerant of MBOA and BOA because it has the capacity to actively detoxify them (1, 3, 4). Genetic and physiological analyses identified two loci, *FDB1* and *FDB2*, involved in this metabolic transformation (1). Mutation at either *fdb1* or *fdb2* conferred sensitivity to the antimicrobials since the compounds could not be metabolized. Given that out of nearly 60 strains of *F. verticillioides* screened for tolerance to BOA only one was sensitive and could not detoxify the compound, we were curious to assess what impact detoxification of these corn metabolites might have on *in planta* associations. Virulence assays performed on genetically characterized strains indicated that detoxification of these antimicrobials was not a major virulence factor and was not necessary for disease development to occur on corn seedlings. Likewise, detoxification was not necessary for endophytic infection of corn seedlings. The possibility that detoxification may constitute a fitness factor enhancing saprophytic colonization of senescent corn tissue needs to be assessed.

However, the virulence assays performed above did indicate that a pathogenicity factor was segregating independently of detoxification. Further investigation indeed supported existence of a single, genetically inheritable locus in *F. verticillioides* controlling pathogenicity toward seedlings of sweet corn hybrid "Silver Queen". This locus is denoted as *FLP1* (*Fusarium verticillioides* lacking pathogenicity). These growth chamber assays involved planting of seed inoculated with a fungal spore suspension. Disease was typically assessed 20 days after planting (DAP) at which point the seedlings are at the V2 to V3 stage of development. Disease symptoms included development of necrotic lesions on leaves 1 and 2 and/or abnormal development of leaf 3. Abnormal development encompassed mild symptoms, such as bleaching of a middle section of the

3rd leaf, as well as much more severe anomalies, including an apparent lack or degeneration of cells lateral to the mid-vein in the middle of the 3rd leaf. The latter resulted in a shorter leaf with a defined zone of missing tissue, and sometimes resulted in the tip of leaf 4 being bound up in leaf 3 causing a tangle-top appearance. A strain produced one or more of these symptoms if it possessed the pathogenic allele at *FLP1*. A strain possessing the non-pathogenic allele caused no symptom development and the plants looked equivalent to the controls.

Spontaneous mutants of *F. verticillioides* defective in their ability to elaborate conidia were isolated from a cross that shared some genetic history with the strains used for the above assessment of pathogenicity. These mutants produced morphologically normal phialides, but instead of blastic development of a conidium, each phialide reinitiated apical growth that resembled a germ tube. This growth appeared to be determinate, and nuclei were not observed in the reinitiated growth. Genetic analysis of this conidiation mutation indicated that a single locus was responsible for the phenotype, and it segregated independently of *FDB1*, *FDB2*, and *FLP1*. Seedling pathogenicity assays were set up with these mutants to evaluate any impact the mutation may have on the ability of the fungi to infect and cause disease. Evaluation of a single tetrad collection of eight strains indicated that pathogenicity, as controlled by *FLP1*, was indeed independent of the conidiation mutation. One set of twin strains with the mutant conidiation phenotype (AEG 3-A3-2 and AEG 3-A3-8) were non-pathogenic while the other set of twins with the conidiation mutation (AEG 3-A3-5 and AEG 3-A3-6) were highly virulent causing severe disease on the corn seedlings. Likewise, the two sets of normal conidiating twins also differed in their pathogenicity (AEG 3-A3-1 and AEG 3-A3-7 were pathogenic, while AEG 3-A3-3 and AEG 3-A3-4 were non-pathogenic). Pathogenicity was assessed 20 DAP, and seedlings were then sampled for endophytic infections. For each treatment 18 seedlings were assessed by taking tissue samples from the middle of the longest leaf, the whorl, and the area just above the mesocotyl node. The conidiation mutants were greatly affected in their ability to endophytically colonize the seedlings. Strains AEG 3-A3-5 and AEG 3-A3-6 were not isolated from any of the tissue samples, yet these two strains were highly virulent resulting in considerable symptom development as described above as well as stunting of plants. Thus, these two strains were able to cause disease despite their apparent inability to infect the corn seedlings. In contrast were strains AEG 3-A3-3 and AEG 3-A3-4, which produced normal phialides, were non-pathogenic, and were quite proficient at colonizing corn plants. The collective results suggest that 1) endophytic infection is not necessary for seedling disease development, and 2) endophytic infections can exist without any detriment to corn seedlings.

Given the evidence for segregation of a pathogenicity factor, what can we deduce about the nature of this factor? The use of the conidiation mutants in the above seedling assays provided some clues. Since that strains AEG 3-A3-5 and AEG 3-A3-6 apparently were not able to infect the corn seedling but were still highly virulent, seedling disease may not be dependent upon fungal infection. Perhaps the disease results from a phytotoxin that is synthesized and secreted by the fungus and ultimately translocated by the plant. Future work will further examine the possibility of a phytotoxin as well as gather mycotoxin data on the various strains involved. A non-pathogenic strain that produces conidia, is effective at endophytic growth, and is also a non-producer of

fumonisins could be useful for examination of competitive exclusion as a means of biocontrol against fumonisin-producing strains.

1. Glenn, A. E., Hinton, D. M., Yates, I. E. and Bacon, C. W. 2001. Detoxification of corn antimicrobial compounds as the basis for isolating *Fusarium verticillioides* and some other *Fusarium* species from corn. Appl. Environ. Microbiol. 67: 2973-2981.
2. Munkvold, G. P., McGee, D. C. and Carlton, W. M. 1997. Importance of different pathways for maize kernel infection by *Fusarium moniliforme*. Phytopathology 87: 209-217.
3. Richardson, M. D. and Bacon, C. W. 1995. Catabolism of 6-methoxy-benzoxazolinone and 2-benzoxazolinone by *Fusarium moniliforme*. Mycologia 87: 510-517.
4. Yue, Q., Bacon, C. W. and Richardson, M. D. 1998. Biotransformation of 2-benzoxazolinone and 6-methoxy-benzoxazolinone by *Fusarium moniliforme*. Phytochemistry 48: 451-454.

RESISTANCE TO *Fusarium* EAR ROT AND FUMONISIN PRODUCTION

Don G. White[1], Michael J. Clements[1], Craig E. Kleinschmidt[1], C.M. Maragos[2] and J.K. Pataky[1]. [1]Department of Crop Sciences, University of Illinois, Urbana, IL; [2]USDA-ARS, Mycotoxin Research Unit, Peoria, IL

Our research objective is to identify high levels of resistance to *Fusarium* ear rot and fumonisin production in corn. A search for sources of resistance is possible due to a collection of approximately 1,600 corn inbreds maintained by the University of Illinois, Department of Crop Sciences. The collection contains inbreds from Canada, Europe, Mexico, northern and central China, India, South Africa and the United States. It also includes a number of lines that were selfed from exotic populations. In 1999, 1589 of the inbreds in the collection were crossed with FR1064. FR1064 is a widely used inbred that is moderately susceptible to *Fusarium* ear rot and fumonisin production. FR1064 is a "stiff stalk" related inbred as are many of the commercially used corn inbreds used as the female parent of corn hybrids grown in the United States. The F_1 crosses between the inbred lines and FR1064 were grown in two replicates at Urbana, IL. The F_1 crosses also were grown in Ponzer and in Winterville, North Carolina. Ears at Urbana were inoculated by injecting 10 ml of a spore suspension down the silk channel at the R1 (silking) growth stage and a second time by injecting 10 ml into the side of the ear at the R2 growth stage. The spore suspension contained approximately 1×10^6 conidia per ml of an equal mixture of three isolates of *Fusarium verticillioides* and three isolates of *F. proliferatum* that had been shown to be the most virulent and the greatest producers of fumonisin in a study done in 1998 (Clements and White, unpublished). Plants in North Carolina were not inoculated because the incidence and severity of *Fusarium* ear rot and production of fumonisin at these locations is typically high.

Severity of *Fusarium* ear rot was rated on the basis of percent of the ear with visible symptoms of *Fusarium*. Fumonisin was determined by an ELISA based on a polyclonal antibody developed by C.M. Maragos. The ELISA protocol allows for detection of greater than 1-2 Fg/g fumonisin in a corn matrix. Lower detection levels (>0.02 Fg/g) are possible in phosphate buffer in the absence of a corn matrix. Modifications to the protocol have allowed for an output of over 400 samples per day.

Ear rot severity of the F_1 crosses ranged from 1 to 59% (mean 8%), 0 to 54% (mean 5%), and 0 to 52% (mean 5%) of the ear rotted in Urbana, Ponzer and Winterville, respectively. Fumonisin concentrations in corn grain ranged from 2 to 180 Fg/g (mean 23 Fg/g), 2 to 163 Fg/g (mean 15 Fg/g) and 1 to 239 Fg/g (mean 13 Fg/g) in Urbana, Ponzer and Winterville, respectively. Twenty-nine unrelated inbred lines were chosen for further evaluation based on grain samples with less than 4 Fg/g fumonisin in all locations evaluated.

In 2001, the 29 resistant selections were evaluated as the resistant parent, the susceptible parent, the F_1, the F_2, and the back-cross susceptible generation. Evaluations were done at Urbana, IL and Haubstadt, IN. Ears were inoculated twice as previously described. From the 29 resistant lines, the best will be selected for future studies of the inheritance of resistance and the identification of quantitative trait loci (QTL) associated with resistance. This research is the first step necessary for eventual incorporation of resistance into widely used commercial inbred lines.

RAPID DETECTION OF SINGLE KERNEL CHARACTERISTICS

Floyd Dowell[1], Tom Pearson[1] and Don Wicklow[2]. [1]USDA-ARS, Grain Marketing and Production Research Center, Manhattan, KS; [2]USDA-ARS, Mycotoxin Research Unit, NCAUR, Peoria, IL

Corn Applications. Recent advances in detection and sorting technology have made it possible to rapidly detect characteristics of single kernels, and to sort kernels based on those measured attributes. Much of this technology is based on near-infrared spectroscopy. We have recently published research demonstrating that we can detect corn kernels with high or low levels of aflatoxin (Pearson et al. 2001), fumonisin (Dowell et al. 2001), and transgenic traits (Kramer et al. 2000) using laboratory NIR systems. We have plans to determine if we can make these determinations when using high-speed sorting systems.

Detection Technology. Single kernel wheat and corn characteristics have been measured manually and automatically using fiber optics and NIR spectrometers. Manual measurements were obtained by collecting spectra from hand-placed kernels. Spectra were obtained by spectrometers manufactured by Ocean Optics (Dunedin, FL) and Perten Instruments (Springfield, IL). Automated measurements were collected on single wheat kernels from a system developed by ERU and marketed by Perten Instruments. This system can process kernels at a rate of 1 kernel/s. We are currently developing a system that includes sorting capabilities, and we plan to adapt the system to handle corn. We have also tested a high-speed system marketed by Satake, Inc (Houston, TX) for removing Karnal bunt from wheat samples, and for removing red wheat from white wheat. These systems can detect and sort single kernels at a rate of 9,000 kg/hr.

Other Applications. We have used this technology to detect other attributes such as: scab damage in wheat (Dowell et al 1999); wheat kernel vitreousness (Dowell 2000); internal insects in wheat (Dowell et al 1998); and attributes of peanuts, sorghum, figs, and blueberries.

Dowell, F. E. Detecting vitreous and non-vitreous durum wheat kernels using near-infrared spectroscopy. Cereal Chem. 77(2):155-158. 2000.

Dowell, F. E., Pearson, T. C., Maghirang, E. B., Xie, F., and Wicklow, D. T. Reflectance and transmittance spectroscopy applied to detecting fumonisin in single corn kernels. Cereal Chem. Accepted June 2001.

Dowell, F. E., Ram, M. S. and Seitz, L. M. Predicting scab, vomitoxin, and ergosterol in single wheat kernels using near-infrared spectroscopy. Cereal Chem. 76(4):573-576. 1999.

Dowell, F. E., Throne, J. E. and Baker, J. E. Automated nondestructive detection of internal insect infestation of wheat kernels by using near-infrared reflectance spectroscopy. J. Econ. Entomology 91(4):899-904. 1998.

Kramer, K. J., Morgan, T. D., Throne, J. E., Dowell, F. E., Bailey, M. and Howard, J. A. Transgenic avidin maize is resistant to storage insect pests. Nature Biotech. 18:670-674. 2000.

Pearson, T. C, Wicklow, D. T., Maghirang, E. B., Xie, F., and Dowell, F. E. Detecting aflatoxin in single corn kernels by using transmittance and reflectance spectroscopy. Trans. ASAE. Accepted May 2001.

A TISSUE-SPECIFIC EXPRESSION CASSETTE FOR INCREASING FUNGAL RESISTANCE AND LOWERING MYCOTOXIN LEVELS IN MAIZE KERNELS

Michael J. Muhitch. USDA-ARS, Mycotoxin Research Unit, National Center for Agricultural Utilization Research, Peoria, IL

The maternal tissues that surround the developing sporophyte acts as the first line of defense against fungal diseases of the seed. In maize, *Fusarium verticilliodes* is present even in the pedicel tissues of asymptomatic kernels. In order to increase the resistance of maize kernels to infection by *F. verticilliodes* and other cob-rotting fungi, a gene expression cassette is being developed to express disease resistance genes within the pedicel and other maternal tissues that are associated with the developing seed. Toward this goal the gene encoding the cytoplasmic isozyme of glutamine synthetase that is strongly expressed in the developing maize kernel has been cloned. This gene, GS_{1-2}, contains a relatively small 5' upstream (promoter) region of approximately 660 bp. Sequence analysis of the promoter reveals putative motifs corresponding to cis regulatory elements involved in maternal expression of anthocyanin genes and also elements involved in the regulation of the expression of genes involved in nitrogen metabolism. A deletion series of the 5' upstream region of maize GS_{1-2} (from –664 relative to the putative transcriptional start site to –72 was fused to the reporter gene GUS and stably expressed in maize plants. Histochemcial analysis of the expression of the –664 "full length" construct revealed GUS to be strongly expressed in the pedicel, the subtending glumes and in the pericarp, but not in the embryo or the endosperm. When the promoter was deleted to –394 bp or shorter, GUS activity was noted in additional kernel and vegetative tissues. The GS_{1-2} promoter will now be used to test the whether expressing antifungal or mycotoxin-metabolizing enzymes within the kernel's maternal tissue does in fact lead to reduced fungal infection. This could result in less yield loss due to fungal disease and to better kernel quality by reductions in grain mycotoxin levels.

ORGANIZATTION AND DISTRIBUTION OF FUMONISIN BIOSYNTHETIC GENES IN *Fusarium*

R. H. Proctor, R. D. Platter, D. W. Brown and A. E. Desjardins. USDA-ARS, National Center for Agricultural Utilization Research, Peoria, IL

Fusarium verticillioides is an ear rot pathogen of maize but is also often present in healthy maize tissue. The fungus can produce fumonisins, a family of polyketide-derived mycotoxins, in infected kernels. Fumonisins have been implicated as the cause of several animal diseases, including cancer in rodents, leukoencaphalomalacia in horses and pulmonary edema in swine. There is also an epidemiological correlation between the consumption of fumonisin-contaminated maize and human esophageal cancer in some areas of the world. We are examining the genetics and biochemistry of fumonisin biosynthesis to identify processes that can be exploited to control fumonisin contamination in maize. Early classical genetic studies defined 3 tightly linked loci, *Fum1*, *Fum2*, and *Fum3*, in natural variants of *F. verticillioides* with altered fumonisin production phenotypes. More recently, we identified a polyketide synthase gene (*FUM5*) that is required for fumonisin production. Sequence analysis of the 75-kb region on either side *FUM5* revealed the presence of 22 additional genes. Northern analysis has revealed that the 14 genes immediately downstream of *FUM5* are co-regulated and that their expression is correlated with fumonisin production. In contrast, the expression of three genes further downstream and five genes upstream of *FUM5* is not correlated with fumonisin production. Disruption analyses to determine whether the co-regulated genes are required for fumonisin biosynthesis are in progress. To date, we have found that two genes are required while three others are not. The predicted translation products of most of the 14 co-regulated genes share similarity with the types of enzymes that are expected to be required for the synthesis of fumonisins. These include cytochrome P450 monooxygenases, fatty acid-CoA ligases, transporters, an aminotransferase, and a carbonyl reductase. Together, these data indicate that we have identified a fumonisin biosynthetic gene cluster.

As an alternative approach to determine gene function, we are currently conducting complementation experiments to determine which genes in the cluster correspond to the three *Fum* loci identified via classical genetics. To date, we have found that the *Fum1* locus and *FUM5* are the same gene (*FUM1*). The *Fum2* and *Fum3* loci confer the ability to hydroxylate the fumonisin backbone at C-10 and C-5, respectively. Therefore, we expect that each of these loci correspond to cytochrome P450-encoding genes in the cluster. We have also begun to determine how widely fumonisin biosynthetic genes are distributed within the genus *Fusarium*. The results indicate that the genes are present in a few relatively closely related species that produce fumonisins.

The identification of fumonisin biosynthetic genes has already facilitated studies on the role of fumonisins in maize ear rot, the use of fumonisin-nonproducing strains as biocontrol agents, and the regulation of fumonisin production. Such studies may provide information that can be used in strategies to eliminate or reduce fumonisin contamination of maize.

Panel Discussion: Fumonisin Contamination of Corn and Development of Cellular, Biological, and Environmental Control Strategies

Panel Chair: Anthony E. Glenn

Panel Members: Bruce Hammond, Gary Payne, Charles P. Woloshuk, Don G. White, Michael J. Muhitch, and Robert H. Proctor

Summary of Presentations: The Second Fumonisin Elimination Workshop was once again held immediately prior to the Aflatoxin Elimination Workshop. The moderator of this session was Jennifer Snyder of the Corn Refiners Association, Inc. The recent expansion of the workshop program to include fumonisins stems from food safety concerns associated with fumonisin contamination of corn. Such contamination results from pre-harvest fungal infection of corn, primarily by *Fusarium verticillioides*. This fungus is widely considered an endophyte of corn plants, is found world-wide wherever corn is grown, and is often the dominant fungus found infecting corn tissues, including kernels. Thus, given its close association with corn and its capacity to produce fumonisin mycotoxins, management strategies to eliminate, or at least significantly reduce levels of infection and production of fumonisins are of obvious concern. This is especially true given the recent release of FDA's "Draft Guidance for Industry" related to fumonisin levels in both human food and animal feed.

Reflective of the growing interest in *F. verticillioides* and its processes of infection and fumonisin contamination of corn, this year's workshop program consisted of four presentations from ARS laboratories, three from university laboratories, and one from Monsanto Company. The presentations outlined the biological complexity of this fungus and its interactions with corn, while identifying potential strategies of control. The reports are summarized below in the order of their presentation.

Bruce Hammond reported on Monsanto's domestic and international field trials examining the utility of their transgenic *Bt*. corn hybrids for controlling development of ear rot and fumonisin contamination. This extra benefit comes in addition to the original targeted season-long control of lepidopteran insect pests, including corn borers and fall armyworm. Their studies have shown reductions in fumonisin levels ranging from 2-3 fold in the United States to 30 fold in France. While there may be some effect of location on the efficacy of fumonisin control, even a modest reduction in contamination could have significant impact on total exposure. While some controversy remains over use of transgenic crops, especially those intended for human consumption, the potential benefit of Bt corn to public health by reducing exposure to fumonisins (and aflatoxins) should continue to receive consideration. With this in mind, Monsanto is initiating further field trials in South Africa, the Philippines, Central and South America, and other regions.

Beyond the use of transgenic *Bt*. corn, no commercial hybrids are available with substantial natural resistance to *Fusarium* ear rot and fumonisin contamination. Traditional management practices, such as time of harvesting and weather monitoring, are still very important for minimizing fumonisin contamination. To fully maximize management strategies, we must have a strong understanding of the basic biology of *F. verticillioides*, including how it infects, its temporal and spatial progression of infection,

how it interacts with the corn plant, and the factors and processes that result in production of fumonisins. Several reports addressed these various aspects.

Gary Payne of North Carolina State University reported on temporal studies of seed infection by *F. verticillioides* and fumonisin accumulation in corn kernels. Their data showed that infection of corn kernels occurred 4-5 weeks after pollination, with fumonisin B1 appearing 1-2 weeks later. They found some variation due to local conditions, but such early infection and fumonisin contamination was surprising. One additional note of interest was that healthy, non-diseased corn often showed high levels of fumonisin contamination. This suggests visual screening for lack of ear rot alone may not be sufficient to reduce fumonisin contamination in some geographical regions. Favorable environmental conditions for fumonisin production and accumulation in kernels may not be the same as conditions favorable for ear rot development. In general, their study did indicate an early harvest of corn with 24% moisture could result in less fumonisin contamination. However, this has to be weighed against the actual environmental conditions and potential for fumonisin contamination and the extra cost involved in drying harvested corn.

Charles Woloshuk of Purdue University reported on his *Fusarium* ear rot and fumonisin contamination survey data for Indiana conducted from 1991-2001. This is part of an overall monitoring program for various ear rotting fungi and mycotoxins, including aflatoxin, ochratoxin, zearalenone, deoxynivalenol, and fumonisin. His data indicated that in the first year of the survey there was high incidence and severity of *Fusarium* ear rot and fumonisin contamination, but starting in 1992 the incidence and severity has declined dramatically. This decline is not indicative of an overall general decrease in ear rot diseases since there have been outbreaks caused by other fungi. The cause for the decrease in *Fusarium* ear rot and fumonisin production may be due to a number of factors, including weather patterns and insect pressures.

Fusarium verticillioides can infect corn plants at various stages of development, but since the fungus infects developing corn kernels, it is quite often seed transmitted. This transmission means the fungus is strategically positioned to infect the new seedling upon seed germination. Seedling disease can be a problem with some corn varieties, but what is also of interest is the establishment of symptomless infections (i.e., endophytic infections) in the corn plant that can lead to systemic growth throughout the developing plant and potentially infection of the newly developing kernels. As has already been mentioned, this symptomless infection can be of concern due to fumonisin contamination without any outward signs of disease.

From the ARS laboratory in Athens, Georgia, Anthony Glenn reported on recent studies assessing seedling infections and disease development. Utilizing spontaneous mutants of *F. verticillioides* that are unable to produce conidia, they have addressed the role of conidia in establishing plant infections and the impact on seedling disease development. The aconidial mutants, defective at a single genetic locus denoted as *FPH1*, were greatly attenuated in their ability to infect corn seedlings. Despite this lack of infection, some of the aconidial mutants were still able to cause disease symptoms on corn seedlings. Further examination indicated that another genetic locus, *FLP1*, was responsible for pathogenicity. The collective results suggest endophytic infections can exist without any detriment to corn seedlings (and mature plants) and that endophytic infection is not necessary for seedling disease development. Whether any correlation

exists between seedling disease and ear rot development needs to be examined. These results are intriguing given the potential use of a natural strain of *F. verticillioides* as a biological control that does not produce fumonisins and does not cause disease, but does produce conidia and does effectively colonize corn plants.

In an effort to address the current lack of commercially available corn hybrids with natural resistant to *Fusarium* ear rot and fumonisin contamination, Don White of the University of Illinois reported on recent genetic crosses utilizing 1589 inbreds from various populations, including some exotics. These were crossed with FR1064, a widely used moderately susceptible inbred with the preferred "stiff stalk" agronomic characteristic. Similar to observations made by Dr. Payne above, Dr. White noted that some corn lines or individuals were symptomless but still contained high levels of fumonisins. Twenty-nine unrelated inbred lines were chosen for further evaluation because they had grain samples with <4 : g/g fumonisin in the three locations evaluated. Given that 29 unrelated inbreds were identified, it's clear that resistance is present in a number of economically viable genetic backgrounds. This collection will hopefully prove very valuable for integration of resistance into widely used commercial inbred lines.

Floyd Dowell, from the ARS laboratory in Manhattan, Kansas, was unable to present his report due to sudden events, but his collaborator and co-author, Don Wicklow from the ARS laboratory in Peoria, Illinois, gave a brief synopsis of their work. Using NIR spectroscopy and high-speed sorting systems, their work has facilitated advancement of detection and sorting technologies based on rapidly detecting various characteristics of single kernels, including contamination by mycotoxins such as aflatoxins and fumonisins. The initial system was developed for wheat but is being adapted for corn and could prove valuable for reduction of fumonisin contamination of foods and feeds.

The final two reports come from ARS laboratories in Peoria, Illinois. First, Michael Muhitch reported on his approach to specifically express disease resistance genes within the pedicel and other maternal tissues of developing corn kernels as a means to control fungal infection and mycotoxin accumulation. These tissues are of interest since they are the first lines of defense against fungal infections. To develop this approach, the gene for a glutamine synthase that is strongly expressed in developing kernels was cloned. The promoter region of this gene was fused to the GUS reporter gene. This promoter was found to control strong expression of GUS in the pedicel, subtending glumes, and pericarp. With this demonstrated activity and localization using GUS, the next phase is to use the promoter to express antifungal or even mycotoxin-metabolizing genes in these tissues. Such localized strong expression of these enzymes may reduce the level of fungal infection in kernels and also reduce fumonisin contamination.

Robert Proctor presented the second report from Peoria. Over the past few years, Dr. Proctor and the other members of his group have made significant progress toward a substantial understanding of the genetic and biochemical processes involved in fumonisin biosynthesis. Building on traditional Mendelian genetic studies of fumonisin production, they have identified a fumonisin biosynthetic gene cluster in *F. verticillioides* that contains at least 23 genes, including the polyketide synthase gene *FUM5*. Expression studies showed that 14 of the genes are co-regulated and are also correlated with fumonisin production. Eight other genes are not correlated with fumonisin production.

Disruption studies on the co-regulated genes are underway to assess their requirement for fumonisin biosynthesis. Using *FUM5* and a few other genes as probes, they have also screened other *Fusarium* species to assess the taxonomic distribution of these fumonisin biosynthetic genes. So far, the genes appear to be present only in a few relatively closely related species that produce fumonisins. As was discussed at the 2000 Fumonisin Elimination Workshop, a more complete understanding of fumonisin biosynthesis at the molecular and biochemical levels may result in identification of targets for effective intervention of fumonisin production. Such intervention could involve biotechnology similar to that being developed by Dr. Muhitch discussed above.

Summary of Panel Discussion: A common theme emerging from the reports was the impact that environmental conditions can have on the severity of fumonisin production and contamination. Discussion began with a comment from Dr. Neil Widstrom concerning how current data suggest the environmental conditions that influence fumonisin production are not as clearly defined as once thought. Defining specific seasonal parameters, for example temperature and rain (amount and timing), that result in high levels of fumonisin contamination is becoming more difficult since geographical location appears to have a significant impact. Overall fungal population structure, frequency of fumonisin-producing fungi in that population, and their inoculum levels were discussed as contributing factors in combination with environmental conditions. The discussion also focused quite heavily on the issue of some symptomless infections having significant levels of fumonisin contamination. Initial discussion focused on whether such symptomless contamination was simply a fluke or perhaps a subject of considerable concern. If symptomless contamination is found to be a consistent phenotype, what are the factors contributing to mycotoxin production? Fungal biomass within corn tissues was discussed relative to its metabolic activity and interactions with the corn host. Identification and utilization of resistant genetic corn stock were stressed as objectives necessary for management and control of *Fusarium* infection, ear rot development, and fumonisin contamination, especially given the close association between corn and the endophytic *F. verticillioides*. Also discussed was plant gene expression in response to environmental stresses and how *F. verticillioides* is in turn responding. Does the fungus respond to a stressed host plant in such a way to benefit itself, or does the fungus also suffer stress and therefore responds to alter its own microenvironment? How would either response impact mycotoxin production? Clearly much can still be learned about the interactions of *F. verticillioides* with its host and the impact of environmental conditions. Such knowledge in combination with resistant corn lines could greatly facilitate management of fumonisin contamination.

EVALUATION OF FOOD-GRADE DENT CORN AND SWEET CORN HYBRIDS FOR FUMONISIN PRODUCTION

C.E. Kleinschmidt[1], J.K. Pataky[1], C.M. Maragos[2] and D.G. White[1]. [1]Department of Crop Sciences, University of Illinois, Urbana, IL; [2]USDA-ARS Mycotoxin Research Unit, Peoria, IL

Fusarium ear rot of corn caused by *Fusarium verticilliodes* (syn = *F. moniliforme*) and *F. proliferatum* is of concern due to production of the mycotoxin fumonisin B1. The most effective control of fumonisin in corn-based food products is by growing corn hybrids that are resistant to *Fusarium* ear rot and fumonisin production. The objectives of this study were to survey a large number of commercial, food-grade hybrids and sweet corn hybrids for susceptibility to *Fusarium* ear rot and fumonisin production and to identify hybrids with the highest levels of susceptibility.

The experiment was a randomized complete block with two replications. Experimental units were rows of hybrids. One row (plot) each of 68 food-grade dent corn hybrids were randomized within each replication. One row each of 20 sweet corn hybrids were randomized and then grouped together within the block of food-grade dent corn hybrids in each replication.

Inoculum was prepared from a mixture of three isolates each of *F. verticillioides* and *F. proliferatum* that had been selected on the basis of ear rot severity and fumonisin production. All plots were inoculated with 10 ml of the spore suspension injected through the husk leaves into the side of the ear one week after anthesis. Primary ears of food-grade dent corn hybrids were harvested at approximately 18% grain moisture and rated for percentage ear rot on a 5% increment scale. Ears were dried to approximately 14% grain moisture and shelled. The grain from each plot was ground and analyzed for fumonisin concentration with a competitive direct enzyme-linked immunosorbent assay (CD-ELISA). Primary ears of sweet corn hybrids were harvested 10 days after inoculation. Each plot was rated for ear rot on a 0 to 5 scale, with 0 being no visible rot and 5 being severe rot. Sweet corn kernels were removed from the freshly harvested ears to represent a fresh market sample and then frozen as 25g subsamples. Subsamples were analyzed for fumonisin content with a CD-ELISA.

Ear rot differed significantly among food-grade dent corn hybrids ($P < 0.0001$) and among sweet corn hybrids ($P = 0.0065$). Fumonisin production differed significantly among food-grade dent hybrids ($P < 0.0001$), but not among sweet corn hybrids ($P = 0.0725$). Ear rot ranged from 1 to 52% of the total ear rotted. Fumonisin ranged from 3.9 to 254.6 ppm, 29.5 to 162.4 ppm and 3.5 to 252.4 ppm, for white, blue and yellow food-grade dent corn hybrids. Hybrids with low fumonisin concentrations in grain were identified among white and yellow food-grade dent hybrids. None of the blue food-grade dent hybrids were highly resistant to fumonisin production or *Fusarium* ear rot. Pearson's correlation coefficient between *Fusarium* ear rot and fumonisin production for food-grade hybrids was $r = 0.83$ ($P<0.0001$).

Fumonisin ranged from 1.4 to 5.6 ppm for sweet corn hybrids. Fumonisin levels detected in the sweet corn hybrids were high enough for concern. This study is being repeated in 2001 at the University of Illinois. Additionally, selected hybrids with low and high amounts of fumonisin production are being grown in three locations in Illinois to

compare levels of naturally occurring *Fusarium* ear rot and fumonisin production with levels resulting from inoculation. If inoculation is a reliable method of identifying hybrids with high levels of fumonisin, additional food-grade hybrids will be evaluated.

EFFECT OF INSECT DAMAGE ON *Fusarium* EAR ROT AND FUMONISIN CONCENTRATION IN *Bt.* AND NON-*Bt.* CORN HYBRIDS

M.J. Clements[1], K.W. Campbell[2], D.G. White[1], C.M. Maragos[3] and C. Pilcher[2]. [1]Department of Crop Sciences, University of Illinois, Urbana, IL; [2]Monsanto Inc., Monmouth IL; [3]USDA-ARS Mycotoxin Research Unit Peoria, IL

Bt. events may reduce *Fusarium* ear rot and fumonisins in corn by minimizing kernel damage from the European corn borer (ECB), *Ostrinia nubilalis*. An experiment in 2000 to evaluated the effects of damage from ECB and corn ear worm (CEW), *Helicoverpa zea*, on four *Bt.* corn hybrids and their nontransgenic, near-isogenic lines. Insect treatments included infestation with ECB larvae (i) or CEW larvae (ii) and a control (iii). Disease treatments included inoculation with a spore suspension of three isolates of *F. verticillioides* (syn = *F. moniliforme*), and *F. proliferatum* in combination with insect treatments. Fumonisin levels ranged from 1 to 210 ug/g. *Fusarium* ear rot ranged from 0 to 57% of the ear rotted. Disease treated and control plots had similar levels of ear rot (P=0.1774), but significantly different fumonisin concentrations (P=0.0064), with *Fusarium* inoculated plots averaging 79% more fumonisins than controls.

Grain from *Bt* hybrids tended to have lower fumonisin concentrations than grain from non-*Bt* hybrids. Highly significant differences (P=0.0002) in toxin levels were observed between *Bt* and non-*Bt* hybrids only in plots that were not inoculated with *Fusarium*. *Bt* and non-*Bt* hybrids were significantly different for ear rot in *Fusarium* inoculated and uninoculated plots (P=0.0361), with *Bt* hybrids averaging 19% less rot than non-*Bt* hybrids. Non-*Bt* hybrids had significantly more early (P=0.0002) and late season (P=0.0004) ear damage from CEW than *Bt* hybrids. Non-*Bt* hybrids and *Bt* hybrids averaged 3.1 and 0.4 on the Widstrom scale for early season ratings and 1.9 and 0.6 for late season ratings, respectively. Highly significant differences also existed between non-*Bt* hybrids and *Bt* hybrids for leaf damage from the ECB. Non-*Bt* hybrids averaged 2.4 on the Gutherie scale, while *Bt* hybrids averaged 0.

Fumonisin levels in grain harvested from insect infested or un-infested plots were similar (P=0.0754), however infested plots ts averaged 36% more ear rot than controls (P=0.0257). Severity of *Fusarium* ear rot was not significantly (P>0.05) different between ECB and CEW infested plots. Correlations between ear rot and fumonisin levels within ECB, CEW and control plots were highly significant (P<0.01) at r = 0.92, 0.76, and 0.20, respectively.

One pair of near-isogenic lines, Dekalb brand Rx697 and Rx697YG, had consistently lower *Fusarium* ear rot and fumonisin concentrations (Fishers LSD, " = 0.01) than the other near isogenic hybrid pairs examined in this study. This agrees with the observation that background hybrid genetics confound the effects of *Bt* transgenes. *Bt* hybrids that express Cry1A(b) proteins in kernels have the potential to reduce the severity of *Fusarium* ear rot by reducing insect feeding from ECB and CEW. Though *Bt* transgenics had some influence in lowering fumonisin levels during 2000, this study is being repeated over the 2001 growing season to study the effect from transgenic hybrids in additional environments.

INSTABILITY OF N-ACETYLATED FUMONISIN B1 (FA1) AND THE IMPACT ON INHIBITION OF CERAMIDE SYNTHASE IN RAT LIVER SLICES

W.P. Norred[1], R.T. Riley[1], F.I. Meredith[1], S.M. Poling[2] and R.D. Plattner[2].
[1]USDA-ARS, Toxicology and Mycotoxin Research Unit, Russell Research Center, Athens, GA; [2]USDA-ARS, Mycotoxin Research Unit, National Center for Agricultural Utilization Research, Peoria, IL

Fumonisin B1 (FB1) is a mycotoxin produced by *Fusarium verticillioides*. It inhibits ceramide synthase, which is a proposed underlying mechanism responsible for the myriad of toxic endpoints observed. We previously reported that N-acetylation of FB1 prevents ceramide synthase inhibition, but cautioned that impure preparations of FA1 can contain a contaminant with the ability to inhibit ceramide synthase. We now report that FA1 spontaneously rearranges to O-acetylated analogs. These rearrangement products are putative inhibitors of ceramide synthase. Rat liver slices exposed to impure FA1 containing O-acetylated FB1 had sphinganine/sphingosine (Sa/So) ratios of 1.15-1.64. Control slices had Sa/So ratios of 0.07–0.24. Clean-up to remove the O-acetylated FB1 yielded purified FA1, which produced Sa/So ratios in liver slices of 0.08-0.18. After storage for ca. 1 year as either a dry powder in a desiccator, or as a dried film at 4° C, the purified FA1 again contained O-acetylated FB1, and was capable of ceramide synthase inhibition. FA1 was most stable in neutral solution, but in acidic solution the equilibrium shifted towards the O-acetylated forms. FA1 in solid form also rearranged, but more slowly than in acid solution. Since FA1 is considerably less cytotoxic than FB1, these results provide additional support for the conclusion that a primary amino group is necessary for both ceramide synthase inhibition and toxicity.

ROLE OF FOLATE IN FUMONISIN B1-INDUCED NEURAL TUBE DEFECTS

J. Gelineau-van Waes, J. Maddox, L. Grabowski, S. Heller and G. Bennett. Center for Human Molecular Genetics, University of Nebraska Medical Center, Omaha, NE

Fumonisin B1 (FB1), a mycotoxin produced by *Fusarium verticilliodes* and *F. proliferatum*, is a common contaminant of corn that has been associated with a variety of diseases in different species. Recent reports suggest that it may also act as a developmental toxicant. The current studies focus on an investigation of FB1 and its interaction with folate transport systems in the induction of neural tube defects (NTDs). Experimental evidence indicates that exposure to FB1 may adversely affect folate uptake, potentially compromising cellular processes dependent on this vitamin. Since maternal folate deficiency has been causally linked to birth defects, NTDs unexplained by other known risk factors may be caused by exposure to FB1. Preliminary results in our laboratory show a differential genetic susceptibility to FB1-induced NTDs in different strains of inbred mice when FB1 exposure occurs during a narrow gestational window. In these experiments, we characterize the developmental toxicology of FB1 by examining the gross morphological defects found in embryos from two different murine strains [LM/Bc (sensitive) and SWV (resistant)] subsequent to maternal FB1 exposure. Our preliminary results indicate that FB1 exposure during the early stages of gestation leads to NTDs (exencephaly) in our mouse model, but its incidence is highly strain-dependent, and the timing of FB1 exposure is critical. The ability of FB1 to cross the placenta is demonstrated by injecting pregnant dams with ^{14}C-labeled FB1 and measuring radioactive uptake in embryos, as well as other tissues, including the placenta, liver, and kidney. The interaction between FB1 and folate is investigated *in vivo* by examining the impact of FB1 on ^{3}H-folate uptake in the embryo and the placenta. Additional studies examine the impact of FB1 on ^{3}H-folate uptake in primary embryo cell cultures derived from each of the two murine strains. In both the *in vivo* studies, and the *in vitro* studies, ^{3}H-folate uptake is inhibited by pretreatment with FB1. Since it is has been previously shown that FB1 alters folate receptor function *in vitro*, the ability of supplemental maternal folate to protect against the teratogenic affects of FB1 was also investigated. Maternal folinic acid supplementation by daily oral gavage (50 mg/kg/day) was able to reduce the incidence of FB1-induced NTDs by approximately 50%. It is anticipated that the development of this *in vivo* model system to study FB1-induced teratogenesis will aid in further understanding the mechanism(s) of FB1 toxicology, and its potential interaction with folate in the induction of NTDs.

FUMONISINS AND AFLATOXINS IN PREHARVEST CORN IN SOUTH GEORGIA: A FIVE YEAR SURVEY

Wellington Mubatanhema[1], Zeljko Jurjevic[1], David M. Wilson[1], Neil W. Widstrom[2], Filmore Meridith[3] and Bonnie Evans[1]. [1]Plant Pathology, University of Georgia, Athens, GA; [2]USDA-ARS, Tifton, GA; [3]USDA-ARS, Toxicology and Mycotoxin Research Unit, Russell Research Center, Athens, GA

Corn samples were randomly collected on farms before harvest from 41-43 counties in south Georgia from 1996 to 2000. Analyses of fumonisins ($FB_1+FB_2+FB_3$) and aflatoxins ($B_1+B_2+G_1+G_2$) were carried out using HPLC and the Vicam aflatest fluorometer method respectively. The data was statistically analyzed using SAS. There was a high incidence of fumonisin contamination of corn from the five harvest seasons. In the 1996 samples, 86% of the corn samples had fumonisin concentrations ranging from 0.6 : g/g to 30.2 : g/g with four having concentrations between 0.6 and 1.0 : g/g. In the 1997 samples 63% were contaminated with fumonisins, ranging from 1.0 : g/g to 22.7 : g/g. In the 1998 samples 91% of the samples were contaminated with fumonisins, with concentrations ranging from 1.3 : g/g to 33.3 : g/g. In the 1999 corn samples, 67% of the samples had fumonisin concentrations ranging from 0.1 : g/g to 16.5 : g/g. Fourteen samples had no detectable fumonisins and 30.2 % of the samples had concentrations above 1.0 : g/g. Nineteen of the 2000 samples contained less than 1.0 : g/g of total fumonisins, 15 had less 5 : g per gram and 9 had over 5 : g/g. All of the 1996 samples contained detectable aflatoxins, ranging from 5 ng/g to 430 ng/g, with ten of the samples having an aflatoxin concentration less than 10 ng/g. On the 1997 corn samples, 85% of the samples were contaminated with aflatoxins but only 12% of the samples had aflatoxins concentrations above 10 ng/g. Aflatoxin concentration in contaminated samples ranged from 1 ng/g to 130 ng/g. The 1998 corn samples were all contaminated with aflatoxins, with concentrations ranging from 6 ng/g to 3500 ng/g with 95 % of the samples having concentrations above 10ng/g. All the 1999 corn samples had aflatoxin contamination, with concentrations ranging from 1 to 530 ng/g. Seven samples had aflatoxin concentrations above 10 ng/g. The 2000 crop contained aflatoxins from 0 to 440 ppb of aflatoxin. When data from all five harvest seasons was combined, ear damage was correlated with both fumonisin and aflatoxin content and there was a significant positive correlation between fumonisin and aflatoxin contamination although this was not always the case in the year by year analysis.

FUMONISIN B_1 PROMOTES AFLATOXIN B_1 AND N-METHYL-N'-NITRO-NITROSOGUANIDINE INITIATED LIVER TUMORS IN RAINBOW TROUT

D.B. Carlson[1], D.E. Williams[1], J.M. Spitsbergen[1], P.F. Ross[2], C.W. Bacon[3], F.I. Meredith[3] and R.T. Riley[3]. [1]Oregon State University, Corvallis, OR; [2]USDA- APHIS, Ames, IA; [3]USDA-ARS, Toxicology and Mycotoxin Research Unit, Russell Research Center, Athens, GA.

Laboratory studies have described the carcinogenicity of fumonisin B_1 (FB_1) in rodents and epidemiological evidence suggests an association between FB_1 (a mycotoxin produced by *Fusarium moniliforme*) and cancer in humans. This study was designed to reveal in rainbow trout, a species with very low spontaneous tumor incidence, if FB_1 was (i) a complete carcinogen, in the absence of an initiator; (ii) a promoter of liver tumors in fish initiated as fry with aflatoxin B_1 (AFB_1); and, (iii) a promoter of liver, kidney, stomach, or swim bladder tumors in fish initiated as fry with N-methyl-N'-nitro-nitrosoguanidine (MNNG). FB_1 was not a complete carcinogen in trout. No tumors were observed in any tissue of fish fed diets containing 0, 3.2, 23 or 104 ppm FB_1 for a total of 34 weeks (four weeks FB_1 exposure, two weeks outgrowth on control diet, followed by 30 weeks FB_1 diet) in the absence of a known initiator. FB_1 promoted AFB_1 initiated liver tumors in fish fed $ 23 ppm FB_1 for 42 weeks. A one-week pre-treatment of FB_1 did not alter the amount of liver [^{3}H]-AFB_1 DNA adducts which suggests that short-term exposure to FB_1 will not alter Phase I or Phase II metabolism of AFB_1. In MNNG initiated fish, liver tumors were promoted in the 104 ppm FB_1 treatment (42 weeks), but FB_1 did not promote tumors in any other tissue. Tumor incidence decreased in kidney and stomach in the 104 ppm FB_1 treatment of MNNG initiated trout. The FB_1 promotional activity in AFB_1 initiated fish was correlated with disruption of sphingolipid metabolism suggesting that alterations in associated sphingolipid signaling pathways are potentially responsible for the promotional activity of FB_1 in AFB_1 initiated fish.

INHIBITION OF *DE NOVO* SPHINGOLIPID BIOSYNTHESIS REDUCES EXPRESSION OF P42 MAP KINASE (ERK2) IN LLC-PK$_1$ CELLS

S.S. Rentz[1,2], J.L. Showker[2], F.I. Meredith[2] and R.T. Riley [2,1].
[1]Pharmaceutical and Biomedical Sciences, University of Georgia, Athens, GA; [2] USDA-ARS, Toxicology and Mycotoxin Research Unit, Russell Research Center, Athens, GA

Fumonisin B_1 (FB_1) is a fungal toxin produced by *Fusarium verticillioides*, a common pathogen of corn. FB_1 inhibits ceramide synthase, a key enzyme in the *de novo* sphingolipid biosynthesis and turnover pathways. Free sphingoid bases, sphingoid base metabolites, ceramide and more complex sphingolipids generated in the biosynthesis and turnover pathways modulate many downstream signals including mitogen-activated protein (MAP) kinases. In LLC-PK$_1$ (porcine renal proximal tubule epithelial) cells, FB_1 inhibits cell proliferation, induces apoptosis, and alters cell-cell contact in a time and concentration-dependent manner. The increased apoptosis and decreased cell proliferation can be prevented by ISP-1, an inhibitor of serine palmitoyltransferase, the first and rate-limiting enzyme in *de novo* sphingolipid biosynthesis. However, the downstream signaling pathways that are affected by FB_1 disruption of sphingolipid metabolism are not well understood. The purpose of this study was to determine in LLC-PK$_1$ cells changes in expression of p42 MAP kinase, also called extracellular signal-regulated kinase 2 (ERK2) in response to a FB_1 concentration known to inhibit cell growth and induce increased apoptosis. Significant inhibition of cell growth was first noted after 48 h exposure to FB_1 (50 :M). However, p42 MAP kinase was decreased at 24 h and at all subsequent time-points (48 and 72 h) relative to the concurrent control. FB_1 treatment in the absence of serum further reduced the expression of p42 at 48 and 72 h. In order to determine if decreased p42 expression was due to FB_1-induced elevation in free sphingoid bases, cells were treated with a combination of ISP-1 and FB_1. ISP-1 did not reverse the decreased expression of p42 caused by FB_1. However, ISP-1 alone also caused a decreased expression of p42, indicating that FB_1-mediated changes in expression of p42 could be independent of alterations in sphingoid bases but dependent on *de novo* sphingolipid biosynthesis.

KINETICS AND BINDING OF FUMONISIN IN A MODEL SOIL SYSTEM

L.D. Williams[1,2], C.W. Bacon[2], J.L. Showker[2], F.I. Meredith[2], A.J. Franzluebbers[2], M.A. Smith[1], R.D. Wyatt[1] and R.T. Riley[2]. [1]College of Agriculture and Environmental Sciences, University of Georgia, Athens, GA; [2]USDA-ARS, Toxicology and Mycotoxin Research Unit, Russell Research Center, Athens, GA

Fumonisin B_1 (FB_1) is a water soluble, carcinogenic mycotoxin produced by the fungus *Fusarium moniliforme*, which is parasitic to corn plants. It can be found in the ear, roots, and stalks of plants. It is estimated that 90% of the FB_1 consumed by livestock is excreted un-metabolized. The objectives of this research are to determine 1) the kinetics of FB_1 interaction with soil constituents, and 2) if FB_1 is chemically modified in the soil. Leachate columns were used to determine the movement of FB_1 through soil matrices comprised of washed sand and 0%, 50%, 75%, or 100% Cecil sandy loam soil. The movement of FB_1 was compared to that of bromophenol blue (Bb), a dye that moved freely through the soil columns. FB_1-contaminated corn screenings or extracts containing FB_1 were placed on the surface of the soil columns. When corn screenings were used as the source of FB_1, a rain simulator was attached to the top of the column and water was allowed to saturate the corn screenings (extracting the FB_1) and percolate through the column eluting the FB_1. The 100% sand columns slightly retarded the efflux of FB_1 relative to Bb but did not appear to chemically alter the FB_1. The recovery of FB_1 decreased with increasing concentrations of Cecil sandy loam soil. At 0%, 50%, 75% and 100% Cecil sandy loam, approximately 80%, 60%, 50% and 20% of the FB_1 was recovered in the column leachate, respectively. The FB_1 retained on the 100% Cecil sandy loam column was tightly bound as evidenced by the fact it could not be extracted using acetonitrile:water (1:1). However, approximately 40% of the retained FB_1 was extractable using 5% formic acid:acetonitrile (1:1) indicating that the nature of the interaction between was probably ionic. The result of this study indicates that FB_1 is probably quite stable in the soil environment and suggests that while some is tightly bound, under certain environmental conditions the FB_1 could be released and become biologically available.

RIBOTYPING OF *Bacillus mojavensis* STRAINS FROM DESERT SOILS, USEFUL AS BIOLOGICAL CONTROL AGENTS FOR *Gibberella moniliformis* IN MAIZE

Charles W. Bacon and D. M. Hinton. Toxicology and Mycotoxin Research Unit, Russell Research Center, USDA-ARS, Athens, GA

One strain of *Bacillus mojavensis* was shown to be a natural endophyte, dwelling within the intercellular spaces of maize, providing protection against infections from other endophytic microorganisms such as the fungus *Gibberella moniliformis* Wineland (anamorphic synonyms *Fusarium moniliforme, F. verticillioides*). *Bacillus mojavensis* is a relatively recently described bacterial species that is unique to desert soils and is distinguished by 16S rRNA sequence analysis. The major objective of this research was to determine the usefulness of ribosomal operon ribotyping as a means to differentiate among isolates of *B. mojavensis* from its closely related species, as well as distinguishing species diversity of *B. mojavensis* at the strain level. Another objective included characterizing the nature of antagonism expressed by *B. mojavensis* strains to *G. moniliformis*. An automated ribotyping instrument (RiboPrinter Microbial Characterization System, Qualicon/DuPont, Wilmington, DE) was used to generate several sets of ribotyping patterns. Four ribogroups (or ribotyping pattern groups) were detected among the 14 strains of *B. mojavensis*, and these were clearly distinct from related species. The ribotypes within strains of *B. mojavensis* indicated wide genetic diversity, and no strain had ribotypes characteristic of its desert origin. The study established that all 13 strains of *B. mojavensis*, isolated from major deserts of the world, are genetically diverse, endophytically colonized maize and are antagonists to *F. moniliforme*. The results indicated that ribotyping can be used as a definitive way to categorize desert isolates of *B. subtilis* as *B. mojavensis*-like and that the large degree of genetic diversity expressed by differences in ribotyping of strains of this species might indicate wide applications of this species as a biological control agent of several pests.

14TH ANNUAL AFLATOXIN ELIMINATION WORKSHOP

SESSION 2: CROP MANAGEMENT AND HANDLING, INSECT CONTROL AND FUNGAL RELATIONSHIPS

Moderator: ***David Ramos, Walnut Marketing Board***

SUBSAMPLE PREPARATION OF PISTACHIOS

Thomas F. Schatzki and N. Toyofuku. USDA-ARS, Western Regional Research Center, Albany, CA

Sample preparation for aflatoxin determination in nuts and similar commodities requires an intermediate step following sampling, called subsampling, in which the sample is homogenized before an aliquot is drawn for analysis. While observations indicate that roughly 10% of total variation can be assigned to subsampling, no theoretical or experimental work has been done on subsampling until now. This problem is of particular importance now since US and European labs use different subsampling methods the E.U. is planning on setting a subsampling protocol in November 2001. We have studied the two methods and find agreement between newly developed theory and experiment. Dry homogenization, as practiced in the US, typically be grinding up to 10 kg in a Hobart VCM, can be described as a sampling problem in which the ground small particles are sampled for contaminated ones. The computed coefficient of variation is found to quantitatively agree with theory and depend on the aliquot size, the particle size distribution and the probability of contamination of individual nuts in the overall lot. Slurry homogenization, as practiced in the E.U., done by grinding a water/nut slurry of up to 30L, results in an equilibrium homogenization of the contaminant over all the particles (whether they come from a contaminated nut or not). As a result, there is no subsampling error at all in this case, regardless of the physical state of the particles. Slurry homogenization is also less labor intensive and thus preferable in all cases.

AFLATOXIN CONTROL IN PISTACHIOS: REMOVAL OF CONTAMINATED NUTS, ECOLOGICAL RELATIONSHIPS, AND BIOCONTROL

Mark Doster[1], Themis Michailides[1], Peter Cotty[2], Brent Holtz[3], Walter Bentley[1], Dave Morgan[1] and Lorene Boeckler[1]. [1]University of California, Kearney Agricultural Center, Parlier, CA; [2]USDA-ARS, Southern Regional Research Center, New Orleans, LA; [3]University of California Cooperative Extension, Madera County, CA

The effect of removing the nuts left after harvest on navel orangeworm (NOW) infestation (a major factor in aflatoxin contamination of pistachio nuts) was investigated in two commercial orchards. In one orchard during winter pruning, clusters of nuts were removed (a grower practice to reduce *Botryosphaeria* inoculum), while in the other orchard nuts were removed by poling (similar to the practice done in almond orchards). In the pruning experiment, the pruned trees had approximately the same incidence of NOW (23.3% of the early split nuts were infested) at harvest as the unpruned trees (20.9%). In the poling experiment, the poled trees (nuts removed during winter) actually had a higher incidence of NOW infestation in the early split nuts (51.2%) at harvest than the trees that were not poled (30.0%), probably due to NOW moths coming from outside the orchard.

In 2001 a study was initiated to investigate the infestation by NOW of nuts with no or little shell staining. The incidences of NOW infestation in nuts (from 10 commercial orchards) with no dark stain were 9.5, 2.7, 1.4, and 0.6% for nuts <16, 16-17, 18-19, and >19 mm in length, respectively. Similarly, the incidences for nuts with only slight stain (1-10% of the shell surface) were 23.5, 12.7, 4.9, and 1.9% for the same size classes. These results suggest that size might be used in addition to shell stain to remove infested nuts. Because damage by the citrus flat mite results in cracking of the hulls (which is possibly favorable for NOW infestation), nut samples were collected from an orchard with extensive mite damage and are currently being evaluated.

We thoroughly examined 10 atoxigenic strains of *A. flavus* (selected from 229 isolates obtained from California pistachio orchards) for possible use as biocontrol agents. From these 10 strains, we selected the most promising strain (A564) for application in a research pistachio orchard. On 2 July, wheat seeds infected with this strain and with another strain (A815) were applied at the rate of 41.6 g wheat/tree (equivalent to 10 lbs/acre). Soil collected just prior to application of the wheat had a very low density of *A. flavus* (1.24 cfu/g soil). On 5 October, leaf and additional soil samples were taken and are currently being evaluated.

Also in 2001 we initiated a study of the relationship of several factors that might contribute to aflatoxin contamination in commercial pistachio orchards. Soil, leaf, and nut samples were collected from 10 orchards. The incidence of early split nuts ranged from 0.6 to 6.5%, depending on orchard. The orchards with the highest incidences of early split nuts also had relatively poor hull separation from the shell for the normal nuts. The density of *Aspergillus* sect. *Flavi* on the leaves ranged from 0 to 577 cfu/m^2 leaf area, depending on orchard. The soil and nut samples are still being evaluated.

AFLATOXIN CONTROL IN FIGS: DEVELOPMENT OF RESISTANT CULTIVARS, IDENTIFICATION OF CONTAMINATED FRUIT AND BIOCONTROL

Mark Doster[1], Themis Michailides[1], James Doyle[1], Peter Cotty[2], David Morgan[1] and Lorene Boeckler[1]. [1]University of California, Davis, Kearney Agricultural Center, Parlier, CA; [2] USDA-ARS, Southern Regional Research Center, New Orleans, LA

Research was initiated to determine the effect of rehydrating dried figs on the visibility of bright greenish yellow fluorescence (BGYF), which is characteristic of aflatoxin-contaminated figs. Two types of dried fig samples have been obtained. Samples were collected from the Substandard Fig Pool. In addition, ripe Calimyrna figs were collected from a commercial orchard and inoculated with *Aspergillus flavus* in order to obtain large numbers of contaminated dried figs. Although studies using these figs are still in progress, preliminary results suggest that rehydration does increase the visibility of BGYF.

New fig selections have been developed by a breeding program that is attempting to produce a Calimyrna-type fig that had the advantages of Calimyrna figs but not the disadvantages (high levels of decay, insect infestation, and aflatoxin contamination). The 12 selections remaining in the breeding program all had very low levels of fungal decay for fruit harvested from several orchards in 2000 and 2001. All 12 selections had substantially smaller eye diameter of ripe fruit (0.2 to 1.7 mm, depending on selection) than Calimyrna (5.2 mm). After inoculation of fig shoots with a spore suspension of *A. flavus*, all of the new selections had lower incidences of decay caused by *A. flavus* (means ranged from 0.0 to 45.0%) than Calimyrna (63.3%). For noninoculated figs, as the eye diameter of the new fig selections decreased, the incidence of general fungal decay also decreased ($y=17.4x+7.0$; $r^2=0.392$). These results support the idea that figs with small eyes will have less decay and less aflatoxin contamination than figs with large eyes, such as Calimyrna.

In 2001 we also thoroughly examined 10 atoxigenic strains of *A. flavus* (selected from 410 isolates obtained from California fig orchards) for possible use as biocontrol agents. From these 10 strains, we selected the most promising strain (A815) for application in a research fig orchard. On 5 July, wheat seeds infected with this strain and with another strain (A564) were applied at the rate of 41.2 g wheat/tree (equivalent to 10 lbs/acre). Soil collected just prior to application of the wheat had a very low density of *A. flavus* (0.71 cfu/g soil). On 17 August, we collected noncaprified figs from the soil in the experimental block. More noncaprified figs were colonized by *A. flavus* in the inoculated areas (6.0 and 6.7% colonized for areas inoculated with A564 and A815, respectively) than in the noninoculated areas (0.7%). On 3 October, leaf and additional soil samples were taken and are currently being evaluated.

INSECT MANAGEMENT FOR REDUCTION OF MYCOTOXINS IN MIDWEST CORN FY-2001 REPORT

Patrick F. Dowd, R.J. Bartelt, J.J. Beck, J. Barnett, M.A. Berhow, J.P. Duvick, M.L. Lagrimini, G.A. Molid and D.G. White. USDA-ARS, Mycotoxin Research Unit, NCAUR, Peoria, IL

Plant Resistance: Studies of interactions between resistance mechanisms suggest a functional approach towards selecting resistance genes may be valuable in determining effective and durable multigenic resistance. Studies using cabbage looper (nonhost insect) vs. corn earworm or fall armyworm tested on host plant resistance mechanisms indicate a greater to much greater sensitivity of the cabbage looper to corn pest host plant proteins. For example, activated maize RIP at 1000 ppm killed 71.4% of cabbage looper larvae exposed to it, but no corn earworm larvae. This approach identifies mechanisms that are important in the host (such as corn) for insect resistance, but that have been partially to totally overcome by corn pest insects; such mechanisms may need to be "replaced" for enhanced insect resistance in corn or other plants. Protein studies have identified that proteases are important determinants in relative toxicity of active proteins (such as fungal RIPs), and that apparently nontoxic chitinases can enhance efficacy (through better penetration of the protective gut linings) of acutely toxic proteins such as *Bt* by up to two fold. Major functional "categories" of insect resistance mechanisms include those associated with host location/recognition (odor/color/taste), detoxification of insect produced toxins, inhibition of insect nutrition (lectins, protease and amylase inhibitors), factors that enhance penetration of acute toxins (chitinases/proteases), "toughness" factors that prevent insect feeding (lignin) and acute toxins themselves (*Bt* protein, maize RIP). Single gene products such as peroxidases may act indirectly to produce multifunctional resistance. In addition to previously identified mechanisms of acutely toxic products and antinutritive effects, we now have data from transgenic plant studies that indicate enhanced toughness (10x higher for skins of ripe transgenic compared to wild type tomato skins) and up to 2.5 fold greater prey independent presence of beneficial insects (mainly stilt bugs) in field tests (which may be both odor and color associated).

In other studies, fractionation and purification of secondary metabolites responsible for Tex6 silk resistance to caterpillars and those contributing to peroxidase resistance in transgenic plants is continuing. PCR primers have been produced to clone identified insect resistance proteins and identify peroxidase clones producing isozymes previously identified in multiple hybrid, challenge, and tissue assays previously associated with fungal resistance responses.

Mycotoxin management studies: The number of fields intensively monitored for insects, mold inoculum, ear damage, and mycotoxin levels was increased from 3 to 7 this year. Nearly all fields had two hybrids planted in them. Monitoring of sites indicated a wider variation from field to field in incidence of *Fusarium* colonized anthers/pollen in leaf axils (ranging from 33% to 100%) than last year, which may have been related to rainfall and planting date interaction. Planting dates varied over a 3 week period, and this appeared to influence incidence and timing of European corn borer damage and associated mycotoxin parameters. Early planted fields escaped nearly all of the European corn borer damage during critical kernel development, and nearly all visibly infected kernels were associated

with sap beetle damage at harvest. Late planted fields were heavily attacked by corn borers at critical kernel development times (up to 73% of ears per field overall, 40% in milk to soft dough stage), and the incidence of ears with symptomatically *Fusarium* infected kernels was high (up to 30%) in non-*Bt* hybrids. *Bt* hybrids in corresponding fields had only minor incidence of symptomatically infected kernels, which was primarily associated with sap beetle damage (mycotoxin analyses pending). The computer program predicted that there would be some inoculum of *Aspergillus flavus* present in some areas sufficient for ears to be infected. Although heavy rainfall in some areas appeared to lessen the severity, kernels visibly molded with *A. flavus* were found from a few ears at two different locations. This is the first time any visibly molded kernels have been recovered from this area since the early 1990s, and suggests the computer program is providing information with the right trends, despite the limited data that was available from the midwest initially. Computer predictions of *Fusarium*/fumonisin were highly dependent on insect damage this year, and relative levels reflected relative trends in insect distribution. A new commercial trap was tested in *Bt* sweet corn fields and found to be sufficiently effective in detecting sap beetles to be useful in monitoring, although it somewhat less effective at peak populations compared to ARS-developed traps (means of 6.6 vs. 12.8 dusky sap beetles per trap, respectively). *Bt* sweet corn was virtually free of caterpillar damage, but the non-*Bt* hybrid had 22% infestation by corn borers. As in the past, sap beetle damage still occurred at noticeable incidences in *Bt* compared to non-*Bt* sweet corn (31.9% vs. 44.4%, respectively).

Publications

Dowd, P.F. 2000. Dusky sap beetles (Coleoptera: Nitidulidae) and other kernel damaging insects in Bt and non-Bt sweet corn in Illinois. J. Econ. Entomol. 93: 1714-1720.

Dowd, P.F. 2000. Indirect reduction of ear molds and associated mycotoxins in *Bacillus thuringiensis* corn under controlled and open field conditions: Utility and limitations. J. Econ. Entomol. 93: 1669-1679.

Dowd, P.F. 2000. Relative inhibition of insect phenoloxidase by cyclic fungal metabolites from insect and plant pathogens. Nat. Toxins 7: 337-341.

Dowd, P.F. 2001. A field-based integrated management plan for mycotoxins in midwest corn. 42nd Annual Corn Dry Milling Conference. Peoria, IL. USDA-ARS, NCAUR, Peoria, IL. (abstract).

CORN AFLATOXIN MANAGEMENT SYSTEM (CAMS): ASSESSING RISKS AND A TIMELINE FOR COMPLETION

Neil Widstrom[1], Marshall Lamb[2] and Judith Johnson[2]. [1]USDA-ARS, Crop Genetics and Breeding Research Unit, Tifton, GA; [2]USDA-ARS, National Peanut Research Laboratory, Dawson, GA

The year 2001 was the third year during the last 25 years in which the level of aflatoxin contamination of the South Georgia corn crop was less than 20 ng/g. The low level of 8 ng/g contamination in 2001 is also reflected by an average kernel infection by *Aspergillus flavus* of less than 1%. The average kernel infection by *Fusarium moniliforme* was 15%, and results from testing in progress will reveal if any of the samples have toxic levels of fumonisins.

The corn grower must weigh the importance of two major risks regarding aflatoxin when managing corn production each year: 1) the risk of higher amounts of aflatoxin contamination associated with early planting in efforts to obtain the highest yields, and 2) the risk of potentially higher amounts of aflatoxin contamination, additional yield loss, and reduced grain quality associated with delaying harvest to reduce drying costs.

Risk 1 is the most difficult to assess since planting date adjustments must be made based on long- range forecasts of weather for the growing season that will determine the severity of seasonal contamination. In general, hybrid yields will decrease from 0.3-1.0 bushel/acre, or more, for each day of delay in planting while aflatoxin contamination will decrease by approximately 1% for each day of planting delay.

Risk 2, however, can be minimized by monitoring contamination as drydown occurs to determine if and when harvest must take place to avoid excessive contamination. Ambient temperatures and resistance in hybrids are probably the most critical factors controlling the ultimate aflatoxin concentration in corn grain. Optimal temperature for growth of *A. flavus* is about 33°C while that for corn growth without stress is 31-32°C and growth under stress is 25°C. Aflatoxin synthesis is maximized at 30°C while maximum insect activity occurs at approximately 35°C. Aflatoxin rates of accumulation are genotype dependent, and are greater for susceptible hybrids than for those with resistance to *A. favus* and insects. Typically, when 20 ng/g aflatoxin has accumulated in a resistant hybrid, the accumulation in a susceptible hybrid is on the order of 100 ng/g.

The knowledge base for CAMS has been restructured to better accommodate current and predicted weather data. When flow charts are completed, the software will be developed and tested in house to prepare it for field testing in 2002.

Panel Discussion: Crop Management And Handling, Insect Control And Fungal Relationships

Panel Chair: Themis J. Michailides

Panel Members: Mark A. Doster, Patrick F. Dowd, Thomas F. Schatzki and Neil Widstrom

Summary of Presentations: Moderator of this session was David Ramos from the Walnut Marketing Board. While observations indicate that 10% of total variation in aflatoxin sampling can be assigned to subsampling for aflatoxin analysis, the first experimental work done by T. Schatzki showed that slurry homogenization of pistachios eliminates subsampling error, is less labor intensive, and thus preferable in all cases (Schatzki and Toyofuku). Navel orangeworm (NOW) infestation of pistachios is associated with aflatoxin contaminated nuts. A 2001 study with processor library samples for 10 pistachio orchards showed that NOW incidences in nuts with no dark stain or with very little dark decreased as the length of pistachios increased. Factors affecting size of nuts are being studied. Experiments using atoxigenic *Aspergillus flavus* to displace the aflatoxigenic *A. flavus* were initiated in both a fig and a pistachio orchard (Doster, Michailides et al.). Rehydration of figs increases the visibility of BGYF and could be used to detect aflatoxin contaminated figs. New fig selections have smaller ostioles and develop less *A. flavus* infection (Doster, Michailides et al.). Chitinase combined with *Bt* crystal protein can increase toxicity to insects. However, single gene products may act in such a manner that multifunctional mechanisms are produced. Increased peroxidase activity increased insect resistance in plants. Multifunctional mechanisms due to peroxidase include generation of acute toxins, nutritional stress, composition and/or color which appear to increase attraction to beneficial insects (Dowd, P. F.). The year 2001 was the third year during the last 25 years in which the level of aflatoxin contamination of the South Georgia corn crop was less than 20 ng/g and showed a less than 1% *A. flavus* incidence. However, infection by *Fusarium moniliforme* was 15%. Corn growers must weigh the importance of two major risks regarding aflatoxin yearly. 1) The risk of higher amounts of aflatoxin contamination associated with early planting in an effort to obtain the highest yields, and 2) the risk of potentially higher amounts of aflatoxin contamination, additional yield loss, and reduced grain quality associated with delaying harvest to reduce drying costs (Widstrom et al.).

Summary of Panel Discussion: In response to a question asked by Mike Muhitch on peroxidases of transgenic plants, Pat Dowd mentioned that if tobacco is under water stress, it would wilt; however, if tobacco has a chlorophyll promoter, wilting will not occur. Hua Sylvia asked Themis Michailides and Mark Doster why the incidence of *A. parasiticus* in soil is much higher than that of *A. flavus* but yet either figs or pistachios decay most commonly by *A. flavus* instead of *A. parasiticus*. The answer to this question is difficult because it is unknown why *A. parasiticus* is well adjusted for soil environment but *A. flavus* for plants. Mark Doster responded that even in fig orchards we find more *A. parasiticus* in soil samples and more *A. flavus* in fig fruit. Dr. D. Guzman-de-Pena asked whether there is a good correlation between BGYF and aflatoxin contamination in figs. Mark Doster responded that the correlation is good but not perfect because some BGYF figs will not have aflatoxin and some aflatoxin contaminated figs will show no BGYF. Mark Doster reported

that only very occasionally one can see the BGYF on the outside of the fig, and that it is a problem in separating figs that have BGYF. In order to see the BGYF, you will need to cut the fig. He also mentioned that in Turkish figs the BGYF is usually on the outside of the figs, and they use UV light to make these figs glow, and thus separate them from the figs without BGYF. Peter Cotty questioned how the rehydration helps viewing BGYF. Themis Michailides responded that BGYF is usually in the inside of the fig and what the rehydration does is make the flesh and skin of the fruit more transparent, allowing the viewing of the BGYF. Peter Cotty asked also about contaminated debris/in fig and pistachio orchards and how long they last. Bob Klein of the California Pistachio Commission responded that all depends on the cultural practices. Themis Michailides added that we usually find one year old flowers from pistachio male trees in pistachio orchards. Don White asked Neil Widstrom how many bushels of corn one can afford to lose in order to reduce aflatoxin contamination. Neil Widstrom replied that this is not consistent; it depends who really buys the corn. For instance, if the corn has >20 ppb aflatoxin, you can not sell it for human consumption, but only for animal feed. In either case when a grower plants early or late would take a certain risk of yield loss. Ken Damann asked P. Dowd on how peroxidase is measured in corn. Pat Dowd responded that there is a device called abadometer that measures the resistance which is due to peroxidase/lignification (when cell size is small that is an indication of greater lignification). Greater lignification can reduce damage by hemiptera insects because they can not puncture the plant tissues.

EFFECTS OF HARVEST DATE ON AGRONOMICS AND MYCOTOXIN INCIDENCE OF MAIZE IN THE MID-SOUTH

H. Arnold Bruns and Hamed K. Abbas. USDA-ARS, CG&PRU,
Stoneville, MS

Maize grain in the Mid-South matures during mid-summer. Most of the crop is field dried, as mechanical dryers are not available in most of the area. Warm temperatures during this time facilitate field drying but are also conducive to stalk and ear rots as well as mycotoxin production, especially aflatoxin. A study was conducted in 2000 and 2001 at the Jamie Whitten Delta States Research Center in Stoneville, MS to evaluate the effects of delayed harvest on the agronomic characteristics and mycotoxin incidence of 3 *Bt* and 3 non-GMO maize hybrids adapted to the region. Harvests began 2 weeks after the reaching physiological maturity and continued bi-weekly for up to 10 weeks. Data indicates no adverse affects on yield, lodging, dropped ears or increased mycotoxin levels as harvest is delayed.

DEPENDENCE OF AFLATOXIN IN ALMONDS ON THE TYPE AND AMOUNT OF INSECT DAMAGE

Thomas F. Schatzki and Martin S. Ong. USDA-ARS, Western Regional Research Center, Albany, CA

The aflatoxin distribution of single insect damaged Nonpareil almonds (1999 crop) has been measured. Separate distributions were obtained for pinhole-, insect (feeding)-, and gross damage. Only low level aflatoxin contamination (0.0003 ng/g) was found for pinhole-only damaged nuts. The distributions for insect and gross damage did not differ, but differed significantly from the distribution previously obtained for gross damaged Ne Plus almonds from a different producer [also 1999 crop]. The Nonpareil almond distribution could be explained on the basis of a pre-harvest hull splitting, similar to previous results in pistachios (0-4 weeks vs. 2-6 weeks pre-harvest). The Ne Plus distribution differs in detail from pistachio results and from the Nonpareil results found here. This may indicate additional cultural damage of Ne Plus around harvest time and/or use of different sorting parameters. Aflatoxin lot averages of 31.7 and 3.47 ng/g were obtained for 100% insect damaged Ne Plus and Nonpareil almonds, respectively. (The previous Ne Plus work contained a calculation error, which is corrected here). The distribution functions were used to compute the seller-s risk of non-acceptance of lots in the E.U. To obtain a 95% acceptance rate, aflatoxin B_1 levels of 0.12 and 0.22 ng/g would be required, which would correspond to 3.8% and 1.2% (feeding and gross) insect damage in Nonpareil and Ne Plus almond lots, respectively.

LIBERTY™ HERBICIDE REDUCES AFLATOXIN CONTAMINATION IN CORN

K. E. Damann and K. M. Tubajika. Department of Plant Pathology and Crop Physiology, Louisiana State University Agricultural Center, Baton Rouge, LA

The commercial formulation of Liberty (Aventis CropScience) was previously shown to inhibit *Aspergillus flavus* radial growth, aflatoxin B_1 production in liquid culture and in a kernel screening assay. Results from 2 years (1999 & 2000) of field experiments at two locations indicate significant decreases in aflatoxin contamination after Liberty application. Groups of herbicide-tolerant Liberty Link hybrids and their near-isogenic herbicide sensitive counterparts were tested. Upper ears were pinbar inoculated at approximately 20 days post mid silk, and sprayed with a sub-lethal (7 oz. a.i./A) or a herbicidal (34 oz. a.i./A) rate at approximately 40 or 60 days post mid silk. Best results (2000) were obtained with the high rate applied at 60 days. Aflatoxin decreases across seven Liberty Link hybrids averaged 64% at one location and 70% at the other. Aflatoxin decreases across six herbicide sensitive hybrids averaged 70% at one location and 84% at the other. These results suggest that Liberty is effective in significantly reducing aflatoxin contamination when applied late in the growing season and may allow redemption of a previously unmarketable crop.

SIMPLE, CHEAP CLEANUP PROCEDURE FOR AFLATOXIN QUANTITATIVE ANALYSIS IN MAJOR COMMODITIES BY HPLC

Victor S. Sobolev and Joe W. Dorner. USDA-ARS, National Peanut Research Laboratory, Dawson, GA

Despite recent scientific advances in analytical methodology, the cost of existing methods for aflatoxin analysis used in industry and the aflatoxin research community remains high. Many of the methods currently in use employ proprietary, antibody-based cleanup columns or immunoassays. The advantages of these methods are that they are selective and comply with legislated requirements, but they cost substantially more than traditional minicolumn methods, which lacked the desired selectivity, sensitivity, and reliability. The purpose of this work was to develop a simple, fast, reliable, inexpensive chemical cleanup procedure for quantitative determination of aflatoxins in major aflatoxin-important agricultural commodities using HPLC. Such a method has been developed. Aflatoxins were extracted from a ground sample with methanol-water (80 + 20, v/v), and after a single cleanup step on a minicolumn packed with basic aluminum oxide, aflatoxins were quantified by HPLC equipped with a C_{18} column, a photochemical reactor, and a fluorescence detector. Water - methanol - 1-butanol (1400 + 720 + 25, v/v/v) served as the mobile phase. Recoveries of aflatoxins B_1, B_2, G_1, and G_2 from peanuts spiked at 5.0, 2.5, 7.5, 2.5 µg/kg were 87.2 ± 2.3, 82.0 ± 0.8, 80.0 ± 1.8, and 80.4 ± 2.8%, respectively. Similar recoveries, precision, and accuracy were achieved for corn, cottonseed, almonds, Brazil nuts, pistachios, and walnuts spiked within the range of 2.5 - 150.0 µg/kg, which represent the most common levels of contamination. The quantitation limit for aflatoxin B_1 was 1 µg/kg. The total analysis time for a ground sample (including weighing, extraction, purification, and LC determination) did not exceed 15-17 min (multiple samples can be processed under 10 min). The method did not require a wash solvent or any vacuum or pumping devices. It is solvent- and material-efficient. The HPLC column showed high longevity -- no significant change in the column performance was detected after analysis of over 1100 samples of peanuts and hundreds of samples of cottonseed, corn and tree nuts combined. The minimal cost of the minicolumn allows for substantial savings compared with available commercial aflatoxin cleanup devices.

FATE OF AFLATOXIN IN CORN AFTER "NIXTAMALIZACIÓN"

Aurora Verver y Vargas[1], Magdalena Segura Nieto[1] and Doralinda Guzmán-de-Peña[2]. [1] Departamento de Ingenieria Genética; [2]Laboratorio de Micotoxinas, Unidad Irapuato Centro de Investigación y Estudios Avanzados, IPN, México

Traditional "nixtamalización" is an alkaline process to prepare corn tortillas in México since prehispanic times. Several studies have shown that this process destroys aflatoxin B_1(AFB_1) present in corn. The efficiency of the process in destroying aflatoxins has been reported to be around 85-95%, thus 15% of aflatoxin remain in the corn dough (masa). The idea that the remaining aflatoxin in "masa" will be more toxic when ingested, as tortilla, has been proposed. However, Dollear (1969) reported "the most reactive functional groups for ease of attack by chemical agents are the lactone ring of aflatoxin B_1, B_2, and the two lactone rings of G_1 and G_2. The lactone ring opened by hydrolysis with strong alkalis and subsequent reactions such as decarboxylation or oxidation would be anticipated bringing about reduction in toxicity and carcinogenicity". Because nixtamalizacion is a hot alkaline reaction that reaches pH 14, the aflatoxin present in contaminated corn must under go hydrolysis leaving molecules that could react with the aminoacids from corn protein present in the masa. The present study was undertaken to elucidate if the modified aflatoxin that remains in masa (15%) could be bound to the aminoacids of corn changing their pattern and changing AFB_1 toxigenicity. Thus, corn free of aflatoxin and highly contaminated of the same variety were used to performed traditional "nixtamalizacion". Aflatoxin quantification was done before and after the process. Protein and aminoacid pattern was also determined before and after the nixtamalizacion. Chemical characteristics such as fluorescence, Rf values, were evaluated as well as biological activity on chicks. The results suggest that the presence of high concentration of aflatoxin in corn naturally contaminated does not affect the protein pattern neither the aminoacid pattern even after "nixtamalizacion". The chemical characteristics and the toxicity of the remaining AFB_1 in masa are different than those of the free AFB_1.

This project was supported by CONACYT 28782N/1634.
We acknowledge technical help to Yolanda Rodriguez with HPLC quantification.

THE EFFECT OF PROPOXIDE (PROPYLENE OXIDE) FUMIGATION ON *Aspergillus flavus* AND *Aspergillus paraciticus* AT LOW DOSE

Tom Griffith. Vice President, ABERCO, INC., Seabrook, MD

A recent study conducted by DFA of California demonstrated that a propylene oxide concentration of 0.032oz/ft^3 caused a significant decline in viability of both *Aspergillus flavus* and *Aspergillus paraciticus* when they were exposed to Propoxide for 48hrs at 31°C . This study suggests that treatment of products with Propoxide (propylene oxide) to kill insects may also have the beneficial effect of reducing the viability of toxigenic *Aspergillus* species and post harvest production of aflatoxin. Similar studies will be conducted on other mycotoxigenic molds in the future. Since propylene oxide has been shown to be effective in some soil fumigation applications, it is anticipated that its effect on these molds in the soil will be evaluated.

AFLATOXIN AND CYCLOPIAZONIC ACID CONTAMINATION OF PEANUTS IN BOTSWANA

A. Bupe Siame and Janet U. Laing. University of Botswana, Gaborone, Botswana

Fifty samples of peanuts were purchased from different stores around Gaborone, Botswana. The purpose of the study was to quantify the aflatoxin and cyclopiazonic acid (cpa) contents in peanut products on sale in Botswana in order to help develop government regulations in human food in Botswana. The samples were stored at 4 C and then finely ground for aflatoxin and cpa analysis. Fifty gram samples were extracted with 200 ml of methanol-water (85+15) for aflatoxin analysis. A modification of the AOAC CB method was used for sample cleanup prior to determination of aflatoxin content using TLC or HPLC. Cpa was extracted from 50 gram samples that had been defatted with petroleum ether before extraction into 250 ml of methanol-chloroform (20+80) followed by an base/acid partion. Cpa was determined by TLC. Sixteen of 49 samples contained aflatoxin from 1 to 100 ppb and 11 of 35 samples contained cpa. Five of the 35 samples contained both aflatoxin and cyclopiazonic acid. The four samples with aflatoxin above 20 ppb also had cpa contents above 100 ppb.

A SIMPLIFIED HPLC METHOD FOR FIELD AND RESEARCH SCREENING OF AFLATOXIN IN CORN AND PEANUT

Wellington Mubatanhema[1], David M. Wilson[1], Neil W. Widstom[2] and Corley C. Holbrook[1]. [1]Plant Pathology Research, University of Georgia; [2]USDA-ARS, Crop Protection and Management Research Unit, Tifton, GA

Corn or peanut samples can be extracted with a solvent containing 80% methanol and 20% water. The method uses a ratio of one gram sample matrix to two milliliters of extraction solvent with 1% NaCl added to the sample before extraction. The extracted sample is filtered and five ml of the extract is diluted with 20 ml water and then filtered after one minute. The resulting filtrate is then directly injected into the HPLC system using a reversed phase column and detected using fluorescence detection. The post column enhancement can be either by the KOBRA cell or the PHRED cell. The correlations between the Vicam fluorometric method and this screening by HPLC were excellent for both corn and peanuts. The correlations were above 0.95 for the HPLC and Afla P and Afla B columns for peanut and above 0.92 for the HPLC and Afla P columns for corn. The screening method results were linear from about 10 ppb to above 50,000 total aflatoxins. The use of a simple HPLC method will be useful for screening large numbers of experimental samples and is not intended to be used in a regulatory setting. This inexpensive approach using current HPLC technology could result in improved data collection from field research efforts.

PROCESS OF ELIMINATION: HOW SORTING AFFECTS AFLATOXIN CONTENT AND FUNGI IN ALMONDS

James L. Baker[1], Paul Bayman[1,2], Gary Gray[3] and Noreen E. Mahoney[1].
[1]USDA-ARS, Plant Mycotoxin Research Unit, Western Regional Research Center, Albany, CA; [2]Departamento de Biología, Universidad de Puerto Rico, Río Piedras, PR; [3]Blue Diamond Growers, Modesto, CA

Are commercial sorting systems effective at removing aflatoxin-contaminated almonds and aflatoxigenic fungi from the processing stream? Samples of almonds were removed from the processing stream at five points: 1) inputs, unprocessed kernels from warehouse storage; 2) rejects, kernels rejected by electronic sorting; 3) accepts, kernels that passed electronic sorting; 4) pickouts, kernels rejected by manual sorting; and 5) finished, kernels that passed manual sorting. Twenty-four samples were taken from each processing point during a five-day run of two million pounds of almonds. Aflatoxin B1 and total aflatoxin were measured for each sample. Fungi were isolated from twelve samples at each stage, using both surface-sterilized and unsterilized kernels. The sorting system was effective at removing aflatoxin —no aflatoxin was detected in the finished product. Manual sorting was the only step that resulted in a significant decrease in aflatoxin. Surprisingly, sound, finished almonds with no aflatoxin contained more fungi than the pickouts. Incidence of *Aspergillus flavus* group fungi was not correlated with aflatoxin content and did not decrease during processing. Incidence of *Penicillum* was negatively correlated with aflatoxin levels in unsterilized pickouts. Total aflatoxin content was significantly higher than aflatoxin B1, which may be explained by a relatively high incidence of *A. parasiticus*.

14TH ANNUAL AFLATOXIN ELIMINATION WORKSHOP

SESSION 3: MICROBIAL ECOLOGY

Moderator: ***Phil Wakelyn, National Cotton Council.***

EVALUATION OF INTRASPECIFIC COMPETITION (*Aspergillus flavus* Link) AND AFLATOXIN FORMATION IN SUSPENDED DISC CULTURE

D.T. Wicklow, J.R. Bobell and D.E. Palmquist. USDA-ARS, National Center for Agricultural Utilization Research, Peoria, IL

The ability of two non-aflatoxin producing strains of *Aspergillus flavus* (NRRL A-27668; NRRL 29269) to interfere with aflatoxin production by *A. flavus* NRRL A-27837 was examined using a replacement series with the suspended disc culture method. Individual glass fiber discs, affixed to a pin suspended from the caps of humidified 20 ml scintillation vials, were inoculated by adding 90 :1 of a chemically defined salts (SL) medium containing 5% glucose and including *A. flavus* conidial mixtures in the following proportions (aflatoxin producer: non-producer = 100:0, 80:20, 60:40, 20:80, and 0:100) at a constant total density (1 x 10^4 spores/ml). Reductions in the total conidial density of these strains, by dilution with sterile SL medium, had no significant effect on fungal growth (mycelium dry weight) or aflatoxin. Significant ($p < 0.0001$) reductions in aflatoxin were recorded when NRRL A-27668 or NRRL 29269 represented any proportion of the inoculum mixture. Aflatoxin yield (: g/ml) values were less than expected ($p < 0.0001$) from the input ratios for toxigenic vs. atoxigenic conidial inoculum within the replacement series. Aflatoxin yields were also reduced ($p < 0.001$), with a corresponding increase in fungal growth ($p < 0.001$), when conidia from pairs of the following aflatoxin producing strains, NRRL A-27837, NRRL 26473, NRRL 26474 or NRRL 26491, were mixed in equal proportions. A hypothesis is presented that vegetative incompatibility reactions prompting a compensatory growth response identified as Abarrage formation, suppresses the induction of aflatoxin biosynthesis. The suspended disc culture system provides an efficient means for evaluating the outcome of *A. flavus* intraspecific competition on aflatoxin production *in vitro*.

ROLE OF COMPETITION AND ADVERSE CULTURE CONDITIONS IN PREVENTING THE LOSS OF AFLATOXIN PRODUCTION BY *Aspergillus flavus* DURING SERIAL TRANSFERS

Bruce W. Horn and Joe W. Dorner. USDA-ARS, National Peanut Research Laboratory, Dawson, GA

Aspergillus flavus is genetically unstable when repeatedly transferred in culture. Serial transfers often result in loss of aflatoxin production and in associated morphological changes such as reduced sporulation, proliferation of aerial hyphae and an inability to produce sclerotia. However, degeneration does not readily occur in nature as indicated by the wild-type morphological characters of newly isolated strains and the high percentage of aflatoxigenic *A. flavus* from soil and crops in some geographic regions. In this study, three aflatoxin-producing strains of *A. flavus* were serially transferred using conidia for 20 generations (three independent generation lines per strain) on potato dextrose agar at 30 C. The rate of degeneration was compared to that of cultures grown under adverse conditions (elevated temperature, reduced water activity, low pH and nutrient deprivation) and in the presence of competing fungi (*A. terreus*, *Penicillium funiculosum* and the yeast, *Pichia guilliermondii*). The loss of aflatoxin production over generations varied considerably according to strain and the generation line within each strain. In the strain most sensitive to degeneration on potato dextrose agar, aflatoxin-producing ability was maintained to varying degrees under adverse culture conditions but not when *A. flavus* was competing with other fungi. Examination of single-spore isolates from cultures following serial transfers indicated that conidial populations comprised a mixture of wild-type aflatoxin producers and variant colony types that were low or nonproducers of aflatoxin B_1. Therefore, reduction in aflatoxin production over successive generations in the laboratory may be interpreted on a population level in which selection favors variant nonaflatoxigenic individuals. In populations from nature, adverse environmental conditions may instead select for wild-type individuals and remove variant individuals that are observed only in the laboratory.

BIOLOGICAL CONTROL OF AFLATOXIN CONTAMINATION OF PEANUTS WITH NONTOXIGENIC STRAINS OF *Aspergillus flavus* AND *A. parasiticus*

Joe W. Dorner and Bruce W. Horn. USDA-ARS, National Peanut Research Laboratory, Dawson, GA

Application of nontoxigenic strains of *Aspergillus flavus* and *A. parasiticus* to soil around peanut plants effectively reduces pre- and post-harvest aflatoxin contamination. Further development of this technology has focused on the evaluation of different biocontrol formulations and the effect of biocontrol treatments on peanut seed germination and plant survivability.

Evaluation of Biocontrol Formulations: A simple, economical technique for producing an aflatoxin biocontrol formulation was reported at the 2000 Aflatoxin Elimination Workshop. Briefly, the technique involves the coating and entrapment of spores of nontoxigenic strains on the surface of a small grain, which serves as both carrier and substrate. A three-year study was conducted to determine the effectiveness of this formulation technique compared with the more traditional formulation produced by solid-state fermentation. Formulations tested included solid-state fermented rice, spore-coated rice, and spore-coated barley. There were no significant differences among formulations in: (1) establishing biocontrol fungi in the soil; (2) displacing toxigenic strains in the peanut crop; (3) reducing aflatoxin contamination. Because the new formulation technique eliminates the need for sterilizing and drying the substrate, which is a requirement of solid-state fermentation, and is amenable to rapid, large scale production, it offers distinct advantages over solid-state fermentation for production of biocontrol formulations.

Effect of Treatment on Peanut Seed Germination and Plant Survivability: Peanuts from fields that were either treated or not treated with the nontoxigenic, biocontrol strains were planted the following year to determine if infection by nontoxigenic strains affected seed germination or plant survivability. During crop year 1999, peanuts were subjected to severe late-season drought stress and were stored under temperature and relative humidity conditions conducive for fungal proliferation. After storage, both treated and untreated peanuts were highly colonized by *Aspergillus* species. However, the incidence of nontoxigenic strains in treated peanuts was 96.4% compared with only 3.9% in untreated peanuts. Aflatoxin contamination was reduced by 96.7% in treated peanuts. In crop year 2000, treated and untreated seeds were each planted in 10 replicate plots with 100 seeds per plot. Percent germination, as evidenced by cracking of the soil surface, averaged 77.8% for untreated peanuts and 77.9% for treated peanuts. The emergence of plants averaged 76.0% for both groups, and 99% of those plants survived for at least 21 days in both groups. Results showed that the nontoxigenic strains used for biocontrol were no more pathogenic to peanuts than were toxigenic strains found naturally in peanuts.

AFLATOXIN CONTAMINATION AND AFLATOXIN PRODUCING FUNGI IN SOUTH TEXAS: INITIAL EXPERIENCE

Peter J. Cotty and Ramon Jaime. USDA-ARS, Southern Regional Research Center, New Orleans, LA

Regulatory limitations on the quantity of aflatoxins permitted in foods and feeds exist throughout most of the world. Aflatoxin contamination has long been a concern for the United States cottonseed industry because contamination limits markets and reduces seed value. Such contamination costs the U.S. cotton industry millions of dollars annually. Cottonseed contamination by aflatoxins is a perennial problem in both Arizona and South Texas. Cottonseed is a preferred feed for which dairies pay a premium. Cottonseed must contain less than 20 ppb total aflatoxins to enter the dairy market. Industry monitoring of the cottonseed crop from 1997 to 2000 indicated that the annual percentage of South Texas cottonseed that exceeded 20-ppb total aflatoxins ranged from 22% to 70%. During this period, contamination was most problematic in the upper coast and coastal bend regions.

Examination of the structures of *A. flavus* communities resident in South Texas have revealed considerable geographic divergence with the highly toxigenic S strain composing the majority of communities in some areas. The S strain composed up to 74% and 58% of the *A. flavus* community on ginned seed produced in South Texas during 1999 and 2000, respectively. As in Arizona, the S strain may be an important cause of aflatoxin contamination in South Texas.

Aspergillus flavus is highly variable in aflatoxin-producing ability. Certain atoxigenic strains (strains that do not produce aflatoxins) are being used in Arizona to competitively exclude aflatoxin producers and thus reduce aflatoxin contamination. In order to evaluate potential use of atoxigenic strains of A. flavus in South Texas, field tests were initiated in 2000. Three distinct atoxigenic vegetative compatibility groups (VCGs) of *A. flavus* were isolated from soils and cottonseed in South Texas and evaluated in small-scale (1/2 acre to 1 acre) field tests for ability to displace aflatoxin producing fungi. For each strain, colonized sterile wheat seed was applied to soil beneath the cotton canopy by hand, at a rate of 10 lb. per acre. Compositions of the *A. flavus* communities resident in fields (soil) prior to treatment, on the cotton crops at harvest, and in the fields one year after treatments were assessed with vegetative compatibility analyses. Combined, the three atoxigenic strains composed less than one percent of *the A. flavus* communities resident in soils prior to treatment. All three VCGs moved from the soil-applied formulation to the crop during the four months between treatment and harvest. Combined, the three VCGs averaged 92% of *the A. flavus* resident on the crop at harvest from the 3 field plots. One year after the applications, the applied atoxigenic VCGs combined composed 74% to 95% of the *A. flavus* communities resident in treated plots. This indicates the potential for long-term reductions in the average aflatoxin-producing potential of *A. flavus* communities resident in South Texas soils. Results to date suggest atoxigenic strains have the potential to be useful in South Texas as one component of an aflatoxin management program. However, cottonseed economics in South Texas may reduce the extent to which these aflatoxin management procedures are practical.

THE ARS-ACRPC PARTNERSHIP TO CONTROL AFLATOXIN IN ARIZONA COTTON: CURRENT STATUS

Larry Antilla[1] and Peter J. Cotty[2]. [1]Arizona Cotton Research and Protection Council, Phoenix, AZ; [2]USDA-ARS, Southern Regional Research Center, New Orleans, LA

Strains of Aspergillus flavus that do not produce aflatoxins (atoxigenic strains) have been investigated as biological control agents for the prevention of aflatoxins contamination of several crops since the late 1980s. In 1998, following multi-year commercial field testing of one atoxigenic strain, Aspergillus flavus AF36, the Arizona Cotton Growers Association voted among its membership to pursue large scale application of the atoxigenic strain technology. This decision included a commitment to establish a grower owned manufacturing facility to provide sufficient quantities of AF36 inoculum for statewide treatment of Arizona cotton. The Arizona Cotton Research and Protection Council (ACRPC) were assigned the lead role in this industry effort. ACRPC and the Agricultural Research Service (ARS) of the USDA initiated a partnership to pursue research and development intended to result in practical atoxigenic strain technologies for the reduction of aflatoxins contamination. Goals of this partnership include: 1. Development of area-wide management strategies; 2. Optimization of application and agronomic practices to achieve maximum single season and long-term reductions in the aflatoxins content of Arizona cottonseed; 3. Development of processes that grower cooperatives and other grower-run organizations can use to produce needed quantities of atoxigenic strain material; 4. Compilation of information required by EPA for full registration of Aspergillus flavus AF36. The third year of treatments under this collaboration was completed in 2001 with the treatment of 19,975 acres in four treatment areas extending from the Mohawk and Parker Valleys on the far western edge of Arizona to the Maricopa-Stanfield area in the south central portion of the state.

In 2000 four areas were treated with a total treated acreage of 16,094. Aspergillus flavus communities present on crops and in soils were characterized in treated and control areas in order to assess the extent to which community structure had been altered by applications. A. flavus isolates were subjected to strain identification and vegetative compatibility analyses. Over 6,000 isolates were classified with these techniques to assess the year 2000 treatments. Treatment areas were separated from non-treated control fields by one mile buffer zones. Soil, crop (seed) and air samples provided the basis for program analyses. Commercial toxin test results were requested from gins and cottonseed wholesalers and, on a limited number fields, independent sampling and analysis were performed to confirm the commercial tests. Incidences of AF36 on the 2000 crop showed variable but positive results throughout all treatment regions. Evidence of treatment influences in control areas from treatments in 1999 and 2000 was also detected. In the Roll/Wellton area, 80% of the A. flavus on the crop after ginning was AF36 versus 27% on untreated control fields. Incidences of AF36 were 52-72% on the ginned crop in eastern and northwestern Paloma and 93% in the southwestern portion. The southwest fields were treated by ground with material banded under the canopy. Other portions of Paloma were broadcast by air. Untreated Paloma controls ranged from 11-27% AF36 crop incidence. Northern Maricopa County fields produced treated versus non-treated crop AF36 incidences

of 30% vs 5% (Laveen) and 90% vs 3% (Peoria). In the Maricopa-Stanfield area of Pinal County AF36 crop incidences of 76% (Marathon Farms) and 71% (Ak Chin Farms) compared favorably to an 11% average in untreated controls. The most precise aflatoxins results came from this Maricopa-Stanfield area. Working closely with a key grower and gin personnel, commercial toxin tests were run on seed piles from individual fields comprising a large contiguous block of cotton. Fourteen of seventeen fields tested (82%) were below 20 ppb. All control seed lots were over 100 ppb. According to the grower, the farm had never previously produced clean seed.

The manufacturing process has been steadily increased from laboratory scale to production of 1,200 pounds per batch. During 2001 significant design changes were made to the process and the required equipment is currently being fabricated. It is anticipated that the ARS-ACRPC manufacturing facility will be able to manufacture 6,000 lb of atoxigenic strain material per day by the end of 2002. Registration of Aspergillus flavus AF36 with the U.S. Environmental Protection Agency (EPA) limited 2001 treatments to fewer than 20,000 acres. In 2001 additional safety, environmental, and efficacy data were provided to EPA. Additional tests required by EPA including avian and mammalian toxicity tests are underway at contract laboratories and it is anticipated that these will be completed by early 2002. Pending the results of those tests, full registration of AF36 on cotton in Arizona will be aggressively pursued.

BIOLOGICAL CONTROL OF AFLATOXIN IN ALMOND AND PISTACHIO BY PREHARVEST YEAST APPLICATION IN ORCHARDS

Sui-Sheng T. Hua. USDA-ARS, Western Regional Research Center, Albany, CA

The major aflatoxin-producing fungus, *Aspergillus flavus* occupies a broad ecological niche and can reproduce copiously. The spores of *A. flavus* can be airborne. These spores may infect early split nuts, nuts wounded by insects as well as nuts with cracks that occur during the growing season or are damaged mechanically during harvest. Nuts infected by *A. flavus* are the major source of aflatoxin contamination in pistachios. We have screened several hundred of yeasts to determine whether, if any can prevent the growth of *A. flavus*. A few species of yeasts have been shown to be effective in inhibiting both the growth of *A. flavus* and aflatoxin production (Hua *et al.*,1999, Appl. Environ. Microbiol. 65:2738-2740). We are continuing to isolate yeasts from orchards and screen them for inhibitory activity. This research will facilitate the discovery of suitable biocontrol agents that are effective under a wide range of environmental conditions. The mechanisms of biocontrol are being elucidated using biochemical, microbiological and microscopic techniques. These studies will provide information on determining potentially effective combinations of yeast strains for field application.

Attempts to implement *any* control strategy using microbes necessitates a good understanding of the interactions of biocontrol yeasts with *A. flavus*. We have focused on one particular strain of yeast with *A. flavus*. The effect of this yeast on the growth of *A. flavus* on pistachio flowers, pistachio leaves and almond leaves collected from orchard was evaluated. The plant materials were sterilized by autoclave and used for experiments. We monitor the viable number of spores of *A. flavus* on plant samples sprayed with yeast and without. The numbers of yeast and spore of *A. flavus* on experimental samples were enumerated by counting the colony forming unit (CFU). Spore production of *A. flavus* was reduced by 60-80% on plant samples sprayed with this particular yeast.

The experiments conducted in my laboratory demonstrated that certain strain of saprophytic yeast could modulate *Aspergillus flavus* spore production in leaves and flowers. Field spraying of this effective yeasts on almond and pistachio trees may decrease the population of *A. flavus* in the orchards and thus lower the number of nuts infected by this fungus. The outcome may be a reduction of aflatoxin contamination in the edible nuts. Scientists from AgraQuest were contacted to collaborate on their role in conducting field application of yeast to orchards. This collaboration is now in the planning stage.

PANEL DISCUSSION: **Microbial Ecology**

Panel Chair: Donald T. Wicklow

Panel Members: Larry C. Antilla, Peter J. Cotty, Joe W. Dorner, Bruce W. Horn, and Sui-Sheng Sylvia Hua.

Summary of Presentations: The moderator of this session was Phil Wakelyn of the National Cotton Council. Five presentations in this session examined both fundamental and applied aspects of *Aspergillus flavus* intraspecific competition in the biocontrol of preharvest aflatoxin contamination of corn, cottonseed and peanuts. A sixth report described research on the role of selected saprophytic yeasts in reducing *A. flavus* populations in tree nut orchards.

Don Wicklow presented evidence from a study of intraspecific competition *in vitro* that points to an unexplored mechanism of *A. flavus* competition that may interfere directly with aflatoxin biosynthesis. When conidia from genetically distinct strains of *A. flavus*, each capable of producing milligram quantities of aflatoxin in suspended disc culture (SDC), were mixed in equal proportions and applied to SDC, aflatoxin was often reduced to nondetectable levels (< 10 ppb). Wicklow theorized that the interference with aflatoxin biosynthesis is in response to vegetative incompatibility reactions between intraspecific competitors.

Bruce Horn introduced new work aimed at understanding why *A. flavus* loses its ability to produce aflatoxin when repeatedly transferred in laboratory culture. Single spore isolates from cultures following serial transfer revealed a mixture of wild-type aflatoxin producers and variant colony types that were low or nonproducers of aflatoxin B1. Horn interprets this phenomenon on a population level in which selection in laboratory culture favors variant nonaflatoxin individuals, often showing reduced sporulation, proliferation of aerial hyphae and an inability to produce sclerotia.

Joe Dorner reported on the results of a three year study of the effectiveness of different biocontrol formulations for applying non-toxigenic strains of *A. flavus* or *A. parasiticus* in cultivated peanut fields. The simple coating of living barley grains with spores of nontoxigenic *Aspergillus* strains proved as effective in controlling aflatoxin as more costly procedures combining steam sterilization of grains, solid substrate fermentation, and drying. Dorner also showed that non-toxigenic biocontrol strains are not any more pathogenic to peanuts, as measured by seed germination and seedling survival, than toxigenic strains found naturally in peanuts.

Larry Antilla summarized the goals of the 1998 partnership between the Arizona Cotton Research and Protection Council (ACRPC) and the Agricultural Research Service. Results from areas treated in 2000 included incidences of AF36 in all treated regions (total @16,000 acres treated) including adjacent untreated control fields. Seed piles harvested from 14 of 17 contiguous fields treated with the atoxigenic biocontrol strain AF36, and showing an incidence of 71%-76% strain AF36 on the ginned crop, were below 20 ppb. At the same time, all samples of seeds from control fields where strain AF36 comprised only 11% of the *A. flavus* population, were over 100 ppb. Antilla reported that the successful ACRPC/ARS collaboration was continued in 2001 with the treatment of 19,975 acres.

Peter Cotty examined the aflatoxin problem in cottonseed produced in South Texas and then presented results of field tests to displace aflatoxin producing *A. flavus* strains on cotton plants with three atoxigenic strains of *A. flavus*. Combined, the three atoxigenic strains averaged 92% of the *A. flavus* resident on the crop at harvest from field plots treated with sterile wheat seeds colonized by these atoxigenic strains. Furthermore, one year after the applications, the three atoxigenic strains composed 74% to 95% of the *A. flavus* communities resident in the treated plots. Cotty=s results suggest that atoxigenic strains have the potential to be useful in aflatoxin management in South Texas.

Sylvia Hua outlined progress in identifying saprophytic yeasts capable of interfering with *A. flavus* growth and aflatoxin production in tree nut orchards. One of the effective yeast strains was applied as biocontrol inoculum to plant surfaces and shown to reduce *A. flavus* colonization of almond and pistachio leaves as well as pistachio flowers.

Summary of Panel Discussion: Horn=s presentation stimulated some discussion about the potential for non-aflatoxin producing strains of *A. flavus* to retrieve their ability to produce aflatoxin when exposed to a particular plant extract or set of environmental conditions. However no experimental evidence to demonstrate such a >reversion= to toxigenicity in *A. flavus* could be identified by any of the participants. Cotty noted that sometimes it is not possible to rescue the loss of conidial production in *Aspergillus*. He further observed that isolates belonging to the same vegetative compatibility group or VCG are consistently either aflatoxin producers or non-producers. A question directed to Wicklow asked if he had attempted to pair VCG compatible strains using SDC. Wicklow responded that such experiments were planned for the coming year, with the initial focus on aflatoxin producing strains belonging to the same VCG, as previously determined by K.E. Papa and Bruce Horn. Wicklow noted that Bruce Horn has reported substantially fewer *Aspergillus* sclerotia on agar surfaces inoculated with conidial mixtures of non-compatible strains (= different VCGs) versus compatible strains (= same VCG). Cotty was asked if untreated cotton fields were sampled for AF36 inoculum. He replied that the AF36 is a common strain in Arizona and constitutes 3-5% of the *A. flavus* population on cotton plants. However, the AF36 strain constituted 60-70% of the *A. flavus* isolates from an untreated field adjacent to a field treated with the AF36 strain. In response to a question about what the Arizona cotton growers hoped to achieve over the next 3-5 years Antilla, responded that the first goal of growers is to eliminate aflatoxin in cotton. Within the next two years they hope to increase acreage for application of AF36 biocontrol inoculum and a second goal is to transfer this technology for use in reducing preharvest aflatoxin contamination of corn and peanuts. Dorner was asked about the source and quality of seed peanuts used in his field plot trials. He indicated that there is no restriction on *A. flavus* contamination of seed peanuts. There was considerable interest in the methods used by Hua to observe cytological changes in *A. flavus* hyphae following exposure to a biocontrol yeast or yeast culture filtrate. Hua commented that yeast culture filtrate weakens the *A. flavus* hyphae and may lead to the eventual degradation of the hyphae.

Aspergillus POPULATIONS IN SOIL, INFESTATION OF CORN, AFLATOXIN AND FUMONISIN, SPATIAL VARIABILITY IN A MISSISSIPPI DELTA GROWERS' FIELD

Hamed K. Abbas, R.M. Zablotowicz and M.A. Locke. USDA-ARS, Stoneville, MS

We evaluated the ecology of *Aspergillus flavus*, and contamination of corn by mycotoxins in a study assessing the spatial variability of soil populations of *A. flavus*, infested corn and mycotoxin levels in a Mississippi Delta growers field. A 6-ha field was laid out in 126, 9.2-m ^{2} plots. Soil was sampled after planting in May of 2000, and in March 2001 with populations of *A. flavus* was determined by plating on modified Dichloran Rose Bengal (MDRB), selective media. Corn was harvested in 2000, and the yield, infestation of kernels by *A. flavus* by plating on MDRB. Aflatoxin and fumonisin concentrations were determined by ELISA. Propagule density of *A. flavus* was 251 cfu g^{-1} soil in 2000, while following the corn crop, higher propagule densities were observed (794 cfu per g^{-1}). A moderate degree of spatial structure of *A. flavus* populations were observed in the 2001 sample best described by a spherical model, but populations in 2000 exhibited pure nugget behavior. Infestation of corn kernels by *A. flavus* ranged from 0 to 100% (mean 15% infested kernels), aflatoxin concentration ranged from 0 to 1590 ppb (mean = 57 ppb) and fumonisin concentration ranged from 0 to 14.7 ppm (mean = 1.8 ppm). Levels of kernel infestation and aflatoxin and fumonisin concentration were randomly distributed in the field with no correlation between *A. flavus* infestation, aflatoxin and fumonisin levels. Aflatoxin production was found in 68 and 59% of *A. flavus* isolated from soil in 2000 and 2001 respectively, while 84 % of *A. flavus* isolated from corn kernels produced aflatoxin and was confirmed by TLC and LC-MS. Results indicate that within a single field a wide range of *A. flavus* soil populations, the *A. flavus* kernel infestation, and mycotoxin levels vary widely and is typically random across the field.

DNA FINGERPRINTING ANALYSIS OF VEGETATIVE COMPATIBILITY GROUPS IN *Aspergillus flavus* FROM A PEANUT FIELD IN GEORGIA

C. E. McAlpin[1], D. T. Wicklow[1] and B.W. Horn[2]. [1] USDA-ARS, National Center for Agricultural Utilization Research, Mycotoxin Research Unit, Peoria, IL; [2] USDA-ARS, National Peanut Research Laboratory, Dawson, GA

The ability of a species specific DNA probe pAF28 to correctly match 75 strains of *A. flavus* isolated from a peanut field in Georgia with one of 44 distinct VCGs was assessed. Multiple strains belonging to the same VCG typically produced identical DNA fingerprints with the exception of VCG 17 and VCG 24 which contained strains that showed 83 and 87% similarity, respectively. *Aspergillus flavus* isolates sharing more than 80% of the fragments are recognized as belonging to the same DNA fingerprint group. Each VCG represented by a single isolate produced unique DNA fingerprints. The results provide further evidence that the pAF28 probe is able to distinguish *A. flavus* VCGs based on DNA fingerprints and can be used to predict the approximate number of VCGs in a sample population. The DNA probe also hybridized strongly and displayed multiple and distinct bands with other species in *Aspergillus* section *Flavi*: *A. bombycis*, *A. caelatus*, *A. nomius*, *A. pseudotamarii* and *A. tamarii*. While individual strains representing *Aspergillus* species in section *Flavi* produced DNA fingerprints with multiple bands, the banding patterns could not be used to classify these strains according to species.

GENETIC, NUTRITIONAL AND ENVIRONMENTAL FACTORS AFFECTING AFLATOXIN BIOSYNTHESIS

Jiujiang Yu, Perng-Kuang Chang, Deepak Bhatnagar and Thomas E. Cleveland. USDA-ARS, Southern Regional Research Center, New Orleans, LA

Aflatoxins are known to be toxic and extremely carcinogenic compounds produced by the fungi *Aspergillus flavus* and *A. parasiticus* when they infect corn, cotton, peanuts and treenuts. The threat to human health and economic loss resulting from aflatoxin contamination of crops are significant, and therefore, receive attention worldwide. Understanding the regulatory mechanism of aflatoxin production plays a key role in solving the problem of aflatoxin contamination. An aflatoxin pathway gene cluster consisting of at least 23 genes has been identified within a 80 kilo-base pair DNA fragment in both *A. flavus* and *A. parasiticus*. A positive regulatory gene, *aflR*, for activating pathway gene transcription was located in the middle of this gene cluster. *A. sojae*, a non-toxigenic strain in the *A. flavus* group, a fungus used in industrial fermentations, was found to contain a defective *aflR* gene with a premature stop codon leading to premature termination of 62 amino acids in the carboxyl terminus. Thus, with the absence of the functional regulatory protein, no induction of aflatoxin can occur in this food grade *Aspergillus*, even though the studied aflatoxin pathway genes (*avfA*, *omtB*, *nor-1*, *norA*, *verB*, *avnA*, *omtA*, *cypX*, and *moxY*) appear to be present and functional. In aflatoxigenic fungi, adjacent to the *aflR* gene, a divergently transcribed gene, *aflJ*, was also found to be involved in the regulation of transcription. A gene, *aflT*, encoding a membrane bound protein with homology to antibiotic efflux genes for transporting toxin out of the fungal cell, was discovered and characterized.

A group of four genes that constitute a well-defined gene cluster related to sugar utilization in *A. parasiticus* was identified adjacent to the aflatoxin pathway gene cluster. The expression of the *hxtA* gene, encoding a hexose transporter protein, was found to be concurrent with the clustered aflatoxin pathway genes in aflatoxin-conducive medium. Lipid substrate was shown to support aflatoxin production. Peptone medium supplemented with 0.5% soybean oil induces lipase gene expression and aflatoxin formation.

Nitrogen source plays an important role in aflatoxin production. Asparagine, ammonium nitrate and ammonium nitrite containing media support aflatoxin production, while sodium nitrate and sodium nitrite containing media do not. Studies indicated that nitrate has suppressive effect on aflatoxin production and over expression of *aflR* gene may be required to overcome the negative regulatory effect in the nitrogen control circuit. A nitrogen utilization gene cluster consisting of two genes, *niaD* and *niiA*, has been identified from *A. parasiticus*, *A. oryzae*, *A. niger* and *A. nidulans*. In addition, a nitrogen regulator, *areA,* has been cloned. It has been demonstrated that in the intergenic region between *aflR* and *aflJ* several AreA binding motifs have been identified. The AreA binding could prevent AflR binding. It seems that the aflatoxin formation and nitrogen metabolism are closely linked.

Fungi have the ability to maintain internal pH and respond to the environmental changes. Acidic pH condition (pH 2-4) favors aflatoxin biosynthesis. It is established

that *pacC* is a major transcriptional regulatory factor that is affected by pH. In the promotor region of the regulatory gene, *aflR*, at least one *pacC* binding site has been identified. Under acidic condition, *pacC* binding may indirectly affect *aflR* transcription and so the aflatoxin formation.

High water activity favors spore germination and mycelia growth. However, severe aflatoxin outbreak in corn was documented under hot weather and drought conditions. The mechanism *Aspergillus flavus* infestation in corn under adverse conditions is not well understood. The possible scenarios may include a combination of these factors: a) the plant defense mechanism is weakened under water stress conditions; b) higher insect feeding and associated injuries to plant tissues, thus providing entry opportunities for fungal invasion; and c) more fungal spores dispersed in the air under drier climate conditions.

Plant metabolites also play some role on aflatoxin formation. Lipoxygenases are demonstrated to inhibit aflatoxin production. At certain conditions, *n*-Decyl aldehyde reduces not only fungal growth of *Aspergillus parasiticus* but also aflatoxin production by over 95% compared with control. Octanal reduces fungal growth by 60%, however, increases aflatoxin production by 500%, while hexanal reduces fungal growth by 50%, but shows no effect on aflatoxin production.

A relationship between fungal development (production of reproductive spore and survival structure sclerotia) and aflatoxin synthesis was studied. The fungal morphology was significantly altered and toxin synthesis was inhibited during strain degeneration studies. The effect included the inhibition of expression of the toxin pathway genes, including *aflR*.

This information could be very useful in identification of anti-fungal or aflatoxin-inhibitory factors in host plants and for devising strategies to control aflatoxin contamination through genetic engineering.

CLONING AND EXPRESSION OF A LIPASE GENE IN *Aspergillus parasiticus* AND *Aspergillus flavus* AND RELATIONSHIP WITH AFLATOXIN FORMATION

Jiujiang Yu, Deepak Bhatnagar and Thomas E. Cleveland. USDA-ARS, Southern Regional Research Center, New Orleans, LA

Aflatoxins are notorious carcinogens produced by *Aspergillus flavus* and *A. parasiticus* when these fungi infect cotton, corn, peanuts and tree nuts. The infected crop seeds contain abundant oil and lipids. Lipolytic enzymes produced by fungi, such as lipases, may promote fungal growth and aid fungal invasion and toxin production using oil as the carbon source. We have cloned a gene, *lipA*, encoding a lipase from *Aspergillus flavus* strain 1007 and *A. parasiticus* SRRC 143. The genomic DNA sequences from *A. parasiticus* and *A. flavus* were found to be 1654 bp and 1663 bp, respectively. The corresponding cDNA obtained by RT-PCR contained two intervening sequences of 52 bp near the N-terminal region. The coding region consists of 921 bp, capable of encoding 306 amino acids with a calculated molecular mass of 33.5 kDa. Comparing the *lipA* gene products of *A. parasiticus* and *A. flavus*, only five amino acid differences were found even though several nucleotide substitutions were identified. Comparison of the lipase in *A. flavus* encoded by *lipA* with its homolog in *A. oryzae* encoded by *mdlB* showed that their amino acid sequences were identical.

The expression of the *lipA* in aflatoxin-conducive (GMS for glucose mineral salt) and non-conducive (PMS for peptone mineral salt) media were studied by RT-PCR experiments. No mature *lipA* mRNA was detected from either media. When the GMS and PMS media were supplemented with 0.5% soybean oil or peanut oil, the *lipA* gene was expressed. Two *A. flavus* isolates, wool-1 and wool-2 that have been demonstrated the ability to degrade wool were also included in this study. The genomic and cDNA sequences of the *lipA* gene from these isolates were shown to be identical to the *A. flavus* strain 1007. The expression pattern of *lipA* from these isolates was also the same as that from *A. parasiticus* and *A. flavus*. The mature mRNA was readily detectable in aflatoxin-conducive and non-conducive media as long as the medium was supplemented with 0.5% soybean or peanut oil.

There appears to be a correlation between the expression of lipase gene and the formation of aflatoxins. In the presence of lipid substrate in the growth media (GMS and PMS), the lipase gene is expressed and also aflatoxins are detected. This result demonstrated that the expression of the *lipA* gene is inducible by lipid substrates, and that lipid substrates promote aflatoxin formation. The expression of the *lipA* gene is induced with lipid substrate, while aflatoxin-conducive or non-conducive media did not support its expression. It seems, therefore, that the expression of *lipA* gene is independent of the expression of aflatoxin pathway genes. However, *lipA* gene may be important for the establishment of fungal infection in lipid rich seeds such as corn, cotton, peanuts and treenuts.

CLONING AND EXPRESSION OF AN ESTERASE GENE INVOLVED IN AFLATOXIN BIOSYNTHESIS IN *Aspergillus parasiticus*

Jiujiang Yu, Perng-Kuang Chang, Deepak Bhatnagar and Thomas E. Cleveland. USDA-ARS, Southern Regional Research Center, New Orleans, LA

Aflatoxins are toxic and carcinogenic compounds produced primarily by the fungi *Aspergillus parasiticus* and *A. flavus*. Contamination of agricultural crops by aflatoxins poses not only serious food and feed safety concerns but also causes significant economic losses. Significant progress has been made in understanding the genetics and aflatoxin biosynthesis, particularly with respect to the genes and enzymes involved in the aflatoxin bioconversion process. Within the previously characterized 80 kb aflatoxin pathway gene cluster between *adhA* and *norA* genes, we have identified a gene, *estA1*, encoding an esterase from wild type strain of *A. parasiticus* SRRC 143. This gene was found to be transcribed in the same orientation as those of its neighboring genes, *aflJ*, *adhA*, *norA*, *ver-1*, and *verA* on the gene cluster. The genomic DNA sequence was determined to be 1.5 kb. BLAST search of the GenBank database demonstrated that this sequence has significant homology to over 30 carboxylesterases and thioesterases in broad range of species from *Bacillus subtilis* to *Homo sapiens*. The cDNA, obtained by RT-PCR, contained one intervening sequence of 55 bp within the 1 kb coding region. The *estA1* gene is capable of encoding a polypeptide of 314 amino acids. Outside of the aflatoxin pathway gene cluster, an additional copy of the esterase gene, named *estA2*, was cloned from *A. parasiticus* SRRC 143. The *estA2* gene is located within a partially duplicated aflatoxin pathway gene cluster somewhere in the fungal genome. The nucleotide sequence identity between the *estA1* and *estA2* is 98.4% within the coding region and the amino acid sequence is 97.5%, respectively, with only 8 substitutions within the 314 amino acid residues. No apparent defect was identified in the *estA2* gene and in the esterase enzyme encoded by the second copy of the gene (*estA2*).

The *estA1* gene is a homolog of the *stcI* identified in *A. nidulans* and postulated to encode an esterase for the conversion of versiconal hemiacetal acetate (VHA) to versiconal (VAL) involved in the biosynthesis of sterigmatocystin and dihydro-sterigmatocystin. The enzyme encoded by the *estA1* in *A. parasiticus* showed 49% amino acid identity with that of *stcI*.in *A. nidulans*. Based on the similarity between the *estA1* and *stcI* gene product, it is quite logic to postulate that the enzyme encoded by *estA1* is involved in the conversion of VHA to VAL in the aflatoxin biosynthetic pathway. RT-PCR experiments demonstrated that only the *estA1* gene, which is located within the aflatoxin pathway gene cluster, is expressed; no expression of the *estA2* gene was detected under both aflatoxin conducive and non-conducive conditions. The possible reason for the preferential expression of the *estA1* over the *estA2* gene may be due primarily to the position effect of the gene in the fungal genome, similar to those genes such as *aflR2*, *aflJ2*, *adhA2*, *norA2*, *ver1B*, *omtB2* that have been duplicated.

MOLECULAR AND BIOCHEMICAL CHARACTERIZATION OF *Aspergillus flavus* FROM PISTACHIO FLOWERS

Sui-Sheng T. Hua[1] and Cesaria E. McAlpin[2]. [1]USDA-ARS, Western Regional Research Center, Albany, CA; [2]USDA-ARS, National Center for Agricultural Utilization Research, Peoria, IL

Preharvest contamination of pistachio by aflatoxin is a recurrent problem and occasionally serious outbreaks occur. Strains of *Aspergillus flavus* have been isolated from pistachio flowers in the spring. These isolates may be potential sources of inocula to the fruits and nuts. Therefore the aflatoxin production by these strains was evaluated. One of the important characteristics of *A. flavus* is the variation in pectinase activities among different isolates. Pectinase P2c produced by *A. flavus* has been shown to be a determining factor for fungal aggressiveness in cotton. To genotype strains of *A. flavus* the molecular DNA probe pAF28 has been used in several studies for DNA fingerprinting. It can distinguish between isolates belonging to different vegetative compatibility groups (VCG) as well as in the same VCG. The objective of this research was to conduct an initial assessment of the population of *A. flavus* associated with pistachio flower by these biochemical and molecular criteria. Pistachio flowers were collected from Wolfskill Grant Experimental Farm (U. of Calif. Davis, CA). Strings of flowers were cut into 1 cm pieces, then placed on salt agar (6% NaCl, 15g agar/l) supplemented with 100 mg/ml of chloramphenicol and incubated at 28ºC in the dark. Patches of *A. flavus* grown on the flower pieces were observed using a stereomicroscope. Purified single colonies were was used for experiments. Five :1 of *A. flavus* spores (10^6 /ml) were inoculated in the center of PDA and incubated at 28ºC for three weeks in the dark for examining sclerotia formation. Pectinase activity was measured by staining with 0.05% ruthenium red the fungal culture grown in pectin-agarose plates. The size of the clear zone was measured by a ruler in mm. Aflatoxin content in each isolates grown on PDA was analyzed by high performance liquid chromatography using a Hewlett Packard model 1050 Chemstation. Probe was labeled by using the Digoxigenin Non-radioactive Nucleic Acid Labeling and Detection System. CA 19 is the most toxigenic strain. None of the strain among the 23 isolates produced small sclerotia (S type). Six out of twenty three strains produced large sclerotia and the three toxigenic strains all produced large sclerotia. The molecular probe, pAF28 has been used as a hybridization probe on Southern blots to distinguish the genotypes of the fungal strains based on restriction fragment length polymorphornism (RFLP). The resulting phonogram indicates a very diversified *A. flavus* population in pistachio flowers. Since the probe correlates well with different VCG groups, one can make a preliminary assignment of VCGs to a population by the patterns of their finger prints. CA 2 and CA 21, both are atoxigenic, have very high pectinase activities with a clear zone of 8 mm. But CA 19 has the highest pectinase activity and the most toxigenic strain among the 23 isolates. Analysis of the characteristics of *A. flavus* population in pistachio flowers may provide useful information for developing biocontrol strategies and for predicting the severity of aflatoxin contamination in nuts in a particular year. Long-term monitor of the fungal population can lead to a better understanding of the flow of the aflatoxin genes among strains of *A. flavus* in nature.

THE NEED FOR UNIFORM STANDARDS, METHODS AND REPORTING OF DATA FROM BIOCONTROL STUDIES USING ATOXIGENIC *Aspergillus flavus* AND *A. parasiticus*

David M. Wilson. University of Georgia, Tifton, GA

This is an open request for the industry and scientific community to develop uniform standards for reporting results concerning aflatoxin contamination and fungal incidence in biocontrol studies. The results need to be presented in ppb and the fungal field studies need to be logical and based on the probable fate and persistence of the biocontrol fungi in the environment. The studies need to take into account the agricultural, marketing, storage, manufacturing and consumer environments as well as regulations and human health concerns. Aflatoxin control is a serious challenge for United States farmers. It is a marketing as well as a public health issue and there are no easy solutions. However, it is in no one's best interest for the agricultural research community to use different standards for evaluating the different strategies for aflatoxin management in farm and consumer products. We should not use one set of standards for marketing and utilization and a different set of standards for biocontrol research.

KEYNOTE ADDRESS

STRATEGIES ON MYCOTOXIN REGULATORY ISSUES

Douglas L. Park. Center for Food Safety and Applied Nutrition, Food and Drug Administration, Washington, DC

Control programs set up by the Food and Drug Administration (FDA) for aflatoxin, an unavoidable natural contaminant produced by specific molds that invade a number of feedstuffs and basic foods, provides an example of forces that affect risk assessment and management strategies by a regulatory agency. More recently on an international scale, efforts to establish international food standards for fumonisin, deoxynivalenol, ochratoxin A, zearalenone and patulin as well as for aflatoxins demonstrate the complexity of developing regulations and/or standards designed to protect consumer health and ensure fair trade practices on a global scale. The aflatoxin story shows how FDA responded to: (a) a potential carcinogenic hazard to humans in U.S. commodities, (b) the need to acquire information concerning the nature of the hazard, (c) economic and political pressures that may have been affected by regulatory controls, (d) and the constraints of laws within which the Agency must function. Current FDA regulations for aflatoxins address public health concerns for potential contamination in basic foods, residues in milk, and animal feeds for numerous commodities and applications. Regulatory limits, sampling and analytical procedures, decontamination and/or diversion to less risk uses for contaminated product are components of FDA's mycotoxin control programs. Current efforts by FDA to establish regulatory controls for deoxynivalenol, fumonisin and patulin, will add further insight on the role that safety and risk assessment procedures play in the development of action levels and advisories for mycotoxins.

14TH ANNUAL AFLATOXIN ELIMINATION WORKSHOP

SESSION 4: CROP RESISTANCE – CONVENTIONAL BREEDING

Moderator: *W. Paul Williams, USDA-ARS*

THE IDENTIFICATION OF MAIZE KERNEL RESISTANCE TRAITS THROUGH COMPARATIVE EVALUATION OF AFLATOXIN-RESISTANT WITH -SUSCEPTIBLE GERMPLASM

Robert Brown[1], Thomas Cleveland[1], Zhiyuan Chen[2], Shirley Gembeh[1], Abebe Menkir[3], Steve Moore[4], Daniel Jeffers[5], Kenneth Damann[2] and Deepak Bhatnagar[1]. [1]USDA-ARS-SRRC, New Orleans, LA; [2]Department of Plant Pathology and Crop Physiology, Louisiana State University, Baton Rouge, LA; [3]International Institute of Tropical Agriculture, Ibadan, Nigeria; [4]Dean Lee Research Station, Louisiana State University, Alexandria, LA; [5]International Maize and Wheat Improvement Center, El Batan, Mexico

Identifying kernel traits that contribute to kernel resistance to aflatoxin elaboration requires that maize genotypes resistant to aflatoxin production be obtained from breeders. Several genotypes, identified as resistant in U.S. field trials, have been investigated using the Kernel Screening Assay (KSA), and the KSA in conjunction with other techniques. Maize genotypes from Central and West Africa and from Mexico that demonstrated resistance in the KSA, are given priority in breeding trials in these locations. Kernel treatments involving wounding and pericarp wax removal, led to the discovery that resistance requires a viable embryo and that it can be expressed on both a pericarp and a subpericarp level. These findings led to the discovery that pericarp wax of resistant genotype GT-MAS:gk was active against *Aspergillus flavus*. Recently, resistance-associated phenolic compounds in GT-MAS:gk wax, with efficacy against *A. flavus,* have been identified. These could serve as markers in breeding trials. When *A. flavus* GUS--$-tubulin transformants were employed in the KSA, it was found that kernels initially become infected in the embryo, and that for most resistant genotypes, aflatoxin accumulation follows *A. flavus* infection levels. Another KSA-based study, where pre-imbibed kernels were challenged with fungal inoculum, demonstrated aflatoxin inhibition in known susceptible lines. This has led to a research initiative to identify and characterize resistance-associated proteins (RAPs). Recent investigations indicate that differences in constitutive antifungal protein expression between resistant and susceptible lines contribute heavily to kernel ability to resist infection/aflatoxin accumulation. One such RAP, a 14 kDa trypsin inhibitor, active against *A. flavus* and a number of phytopathogens, was discovered along with its ability to inhibit *A. flavus* "-amylase activity. This may constitute another resistance marker for germplasm development. Discovery of stress-related RAPs, using 2 D PAGE and proteome analysis software, have expanded our previous model of "traits required for kernel resistance". These new discoveries highlight the relationship between drought and aflatoxin accumulation. To take full advantage of these traits, the following research will be conducted: 1) biochemical and physiological characterization of RAPs; 2) comparative pathogenesis and protein studies of drought tolerant and resistant germplasm; 3) QTL mapping of corresponding RAP genes to determine contribution to resistance; 4) microarray analyses of RAP gene expression; 5) plant transformation to confirm the role of RAPs in resistance, and 6) gene and/or protein bioassay development to assist breeders in field screening. These investigations can significantly enhance the creation of commercial maize lines with aflatoxin-resistance.

MOLECULAR GENETIC ANALYSIS OF RESISTANCE MECHANISMS TO AFLATOXIN FORMATION IN CORN AND PEANUT

B. Z. Guo[1], N. W. Widstrom[2], C. C. Holbrook[2], R. D. Lee[3], A. E. Coy[3] and R. E. Lynch[1]. [1]USDA-ARS, Crop Protection and Management Research Unit; [2]Crop Genetics and Breeding Research Unit, Tifton, GA; [3] University of Georgia, Coastal Plain Experiment Station, Tifton, GA

Our research goal is to study and identify factors associated with resistance to aflatoxin contamination in corn and peanut and to identify genes/markers associated with the resistance to drought stress and insects in order to develop strategies for control of preharvest aflatoxin contamination. Field and laboratory screening assays have been conducted to evaluate corn and peanut lines/cultivars (genotypes) for resistance/susceptibility to aflatoxin formation. Marked differences in aflatoxin production were detected among the tested genotypes. Using DD-RT-PCR, gene expression profiles have yielded some interesting information in understanding the molecular mechanisms related to drought-stress and drought tolerance associated with resistance. Differentially displayed cDNA fragments were identified, cloned, and sequenced in corn and peanut. We also identified and sequenced a novel *PLD* gene, encoding a putative phospholipase D in peanut, a main enzyme responsible for the drought-induced degradation of membrane phospholipids. Further studies of these drought-stress related genes are needed to genetically and physiologically characterize theses genes in corn and peanut and to gain a better understanding of their function and relationship with drought-tolerance and prevention of preharvest aflatoxin contamination.

Guo, B. Z., A. Butron, H. Li, N. W. Widstrom, and R. E. Lynch. Restriction fragment length polymorphism assessment of the heterogeneous nature of maize population GT-MAS:gk and field evaluation of resistance to aflatoxin production by *Aspergillus flavus*. Journal of Food Production 65: (in press).

Widstrom, N. W., M. E. Snook, and B. Z. Guo. 2001. Synthesis of chemical defenses in maize silks against corn earworm and their inheritance in the flavonoid pathway. Pages 55-69 in: Insects and Plant Defence Dynamics, T. N. Ananthakrishnan (ed.), Science Publishers, Enfield (NH), USA.

Guo, B. Z., Z. J. Zhang, R. G. Li, N. W. Widstrom, M. E. Snook, R. E. Lynch, and D. Plaisted. 2001. Restriction fragment length polymorphism markers associated with silk maysin, antibiosis to corn earworm larvae (Lepidoptera:Noctuidae), in a dent and sweet corn cross. Journal of Economic Entomology 94:564-571.

Butrón, Ana, R. G. Li, B. Z. Guo, N. W. Widstrom, M. E. Snook, T. E. Cleveland, and R. E. Lynch. 2001. Molecular markers to increase corn earworm resistance in a maize population. Maydica 46:117-124.

Guo, B. Z., R. Li, N. W. Widstrom, R. E. Lynch, and T. E. Cleveland. 2001. Genetic variation in the maize population GT-MAS:gk and the relationship with resistance to *Aspergillus flavus*. Theoretic and Applied Genetics 103:533-539.

Guo, B. Z., Z. Zhang, A. Butron, N. W. Widstrom, M. E. Snook, R. E. Lynch, and D. Plaisted. 2001. Quantitative effects of loci *p1* and *a1* on concentrations of

maysin, apimaysin, methoxymaysin and chlorogenic acid in maize silk tissue. Maize Genetics Newsletter 75:64-66.

Guo, B. Z., N. W. Widstrom, T. E. Cleveland, and R. E. Lynch. 2000. Control of preharvest aflatoxin contamination in corn: Fungus-plant-insect interactions and control strategies. Recent Research Developments in Agricultural and Food Chemistry 4:165-176.

Butrón, A., B. Z. Guo, N. W. Widstrom, M. E. Snook, and R. E. Lynch. 2000. Use of markers for maize silk antibiotic polyphenol compounds to improve resistance to corn earworm. Recent Research Developments in Agricultural and Food Chemistry 4:193-201.

Guo, B. Z., A. Butrón, R. G. Li, Z. J. Zhang, N. W. Widstrom, M. D. McMullen, and R. E. Lynch. 2000. Identification of a gene at the syntenic *sh2-a1* region in maize. Maize Genetics Newsletter 74:64-66.

BREEDING AND MOLECULAR MARKER ASSISTED SELECTION FOR RESISTANCE TO *Aspergillus* EAR ROT AND AFLATOXIN PRODUCTION

D.G. White and Torbert Rocheford. Department of Crop Sciences, University of Illinois, Urbana, IL

Our research objective is to incorporate resistance to *Aspergillus* ear rot and aflatoxin production into commercially usable corn inbreds and hybrids. There are several aspects of the research which are interdependent. Our first step was to identify new sources of resistance, which we have accomplished. Then it was necessary to study the inheritance of resistance in order to clearly identify the most useful sources of resistance. This has been done on several sources of resistance, however, it needs to be done with others. The third component is to identify molecular markers (QTL) associated with resistance. This has been done with the resistant inbred Tex6 in crosses with the susceptible inbred B73. Now, resistance is being crossed into commercially used, agronomically acceptable, inbreds using marker assisted backcrossing.

For the past two summers we have been working with the resistant inbred MI82, in crosses with B73. With this study we are including 449 families of the backcross susceptible self generation. MI82 was selected because it was highly resistant in studies done by Bob Brown and F.S. Chew. F.S. Chew determined that the inbred has a protein that inhibits AFL in the aflatoxin biosynthesis pathway. For this study we also rated BGYF associated with ground samples. This is an attempt to partially quantify the amount of fungal growth and relate it to toxin production. In studies done in 2000, the BGYF rating was positively correlated with the amount of aflatoxin (r=0.75) and with ear rot (r=0.58).

We have identified molecular markers associated with resistance from the resistant inbred line Tex6 crossed with B73. An interval in chromosome five (bin 5.01-02) from Tex6 accounts for 14% of the phenotypic variation and a region on chromosome ten (bins 10.5-07) accounts for 15.1% of the phenotypic variation using PLAB QTL. These two areas of the chromosome also were found in resistant backcross derived lines developed without the use of molecular markers.

We are now using molecular marker assisted backcrossing to move at least a portion of the resistance from Tex6 and resistance from MP313E into a widely used commercial inbred, FR1064. FR1064 has a broad range of use extending from the midwestern United States to Georgia and Texas. We have backcross three derived lines of FR1064 with the segment of chromosome four (bins 4.05-09) that has been associated with resistance from MP313E in research done in Mississippi. Also, we have backcross one families of B73 that have the resistance QTL-s from Tex6 on chromosomes five and ten. This past summer we made crosses between the resistance from MP313E (backcrossed three times to FR1064, approximately 93% FR1064) and Tex6 (backcross once to B73) in order to start to pyramid resistance from both inbreds into FR1064. From an agronomic standpoint the phenotypic appearance of inbred lines with three backcrosses to FR1064 that are carrying the chromosome arm from MP313E look very promising.

In winter nursery and next summer we propose to continue crossing resistance into commercially acceptable inbred lines. Also, we intend to begin studies on another

source of resistance, OH516, which is as resistant as Tex6 in several years of evaluations. We also have entered into a cooperative project with Monsanto in order to incorporate resistance from Tex6 into one of their widely used commercial inbred lines that already has some level of resistance.

Publication: Walker, R.D. and White, D.G. 2001. AInheritance of resistance to *Aspergillus* Ear Rot and Aflatoxin Production of Corn from CI2.@ Plant Dis. 85:322-327

DEVELOPMENT OF PEANUT GERMPLASM WITH ENHANCED RESISTANCE TO PREHARVEST AFLATOXIN CONTAMINATION

C. Corley Holbrook[1], Dave M. Wilson[2], Marshall Lamb[3], C. K. Kvien[2], Baozhu Guo[1], K. T. Ingram[4] and Diane L. Rowland[3]. [1]USDA-ARS, Tifton, GA; [2]University of Georgia, Tifton, GA; [3]USDA-ARS, Dawson, GA; [4]University of Georgia, Griffin, GA

Peanuts become contaminated with aflatoxin when subjected to prolonged periods of heat and drought stress. These environmental conditions are not uncommon in the Southeastern peanut production area. The resulting aflatoxin contamination costs the peanut industry, on average, over $20 million. The development of peanut cultivars with resistance to preharvest aflatoxin contamination (PAC) would reduce these costs. Two requirements are needed before beginning the process of breeding a cultivar with resistance to PAC. First we must have screening techniques that can reliably differentiate genetic resistance from susceptibility. During the coarse of this project we have developed field screening techniques that can measure genetic differences in aflatoxin contamination. The second requirement is genetic variation for resistance. During the coarse of this project we have identified 14 core accessions that have shown at least a 70 % reduction in PAC in multiple environments. We have also identified significant reduction in PAC in peanut genotypes with drought tolerance. These sources of resistance to PAC have been entered into a hybridization program. They have been crossed with cultivars and breeding lines that have high yield, acceptable grade, and resistance to tomato spotted wilt virus (TSWV). Due to the large environmental variation in PAC, it is not feasible to examine these breeding populations until late generations when there is less heterozygosity and adequate seed are available for field testing using multiple replications. We have identified families and individual breeding lines that have relatively low PAC, relatively high yield, and acceptable levels of resistance to TSWV. However, much faster breeding progress could be achieved through the development and use of indirect selection techniques. We are exploring this with studies on mechanisms of resistance to PAC and the relationship between this resistance and drought tolerance.

MAIZE GERMPLASM EVALUATION FOR AFLATOXIN RESISTANCE IN TEXAS

Javier Betrán[1], Tom Isakeit[2], Gary Odvody[2] and Kerry Mayfield[1]. [1] Soil and Crop Sciences Department, Texas A&M University, College Station, TX; [2]Pathology Department, Texas A&M University, College Station, TX

Preharvest aflatoxin (AF) contamination is one of the main limitations for corn production in Texas. At present, there are no elite inbred lines resistant to AF that can be used directly in commercial hybrids. Our objective was to evaluate and compare the response of new and selected potential sources of AF resistance to AF contamination under field conditions. The response of inbred lines per se and in hybrid combinations (test crosses) to aflatoxin contamination was determined in three Texas locations: Weslaco (WE), Corpus Christi (CC) and College Station (CS). The inbreds under evaluation were: CML288, CML289, CML294, CML285, CML 323, CML338, Tx601y, NC300, B104, Tx714, Tx732, FR2128, B97, Tx770, and Tx772 among the yellows; and CML176, CML269, CML311, CML322, CML373, CML379, CML384, CML385, CML343, CML78, Tx114, Tx130, Tx110, Tx807, T39, T35, Y9121 among the whites. These sets of lines represent a wide range of adaptation (tropical, subtropical and temperate), agronomic performance, and plant and grain attributes. Commercial susceptible and resistant checks were included in the trials. The silk channel inoculation technique was used at CS and WE, while in CC, *Aspergillus flavus*-colonized corn kernels were placed on the soil surface between treatment rows around mid-silk stage. Drought and heat stress was induced by late planting and limited irrigation.

Significant differences among the inbreds and hybrids were detected in all the locations. At WE the average AF was 1341.18 ng g^{-1} (range: 115.3 to 2675.0 ng g^{-1}) for white inbreds and 1343.17 ng g^{-1} (range: 452.5 to 2750.0 ng g^{-1}) for the yellow inbreds. The inbreds with less AF were CML176, CML78, and Tx130 among the whites, and CML289, Tx601y, and Tx732 among the yellows. The average AF was 487.85 ng g^{-1} (range: 73.0 to 1312.5 ng g^{-1}) for white hybrids and 530.7 ng g^{-1} (range: 77.0 to 1825 ng g^{-1}) for yellow hybrids. The hybrids with less AF were CML269/CML176, CML379/Tx130, and CML384/CML176 among the whites, and Tx601y/NC300, CML161/CML170 and Tx770/CML325 among the yellows. Inoculated ears had significantly greater AF than non-inoculated ears in the same control plots. Inbreds per se showed more AF than hybrids in both white and yellow grain. The correlation between inbred lines per se and their test crosses was variable depending on the testers. At CC the average AF was 872.70 ng g^{-1} (range: 133.3 to 4800.0 ng g^{-1}) for white hybrids and 1682.2 ng g^{-1} (range: 86.7 to 5633.3 ng g^{-1}) for yellow hybrids. White hybrids were less susceptible than yellow hybrids. Heavy insect infestation and drought stress was observed at CC and there was a strong correlation between secondary traits such as insect ($r = 0.7^{**}$) and ear rot (0.70^{**}) ratings with AF. The correlation between AF at CC and WE for hybrids tested in both locations was non-significant indicating that the environmental component of AF contamination is high.

The most promising inbreds for our conditions were CML176, CML269, CML322, and Tx130 among the whites; and Tx601y, CML289, Tx770 and CML338 among the yellows. Some of these inbreds have shown desirable agronomic

characteristics in Texas. Our future plans are: to evaluate further the best inbreds for AF resistance, to characterize their resistance factors through mating designs and genetic mapping, to combine resistant factors from different resistant sources, to transfer the resistance to elite inbreds parents, and to screen additional elite and exotic germplasm.

FIELD PERFORMANCE OF SEED AND ENDOCARP BASED RESISTANCE TO PREHARVEST AFLATOXIN CONTAMINATION IN ALMOND

Thomas M. Gradziel and Abhaya M. Dandekar. Department of Pomology, University of California, Davis, CA

Earlier research has shown that vulnerability to preharvest aflatoxin contamination of almond [*Prunus dulcis*] occurs during a well defined period of fruit development and is associated with insect damage to the kernel. High levels of aflatoxin control have been achieved in genotypes selected for an integrated insect/fungal resistance within almond mesocarp, endocarp and seed tissue. Large scale field testing in northern, central and southern California growing regions continue to demonstrated effective control from 1998-2001. Individual resistance mechanisms, particularly endocarp histogenesis, have been analyzed using NMR and light microscopy, and inheritance studies. The developing almond kernel is protected from insect and disease damage by a fleshy mesocarp and lignified endocarp. At maturity, the mesocarp or hull desiccates and splits leaving the lignified endocarp or shell as the remaining barrier to insect and disease. California almonds typically possess a moderately lignified 'paper' shell rather than the stony, peach-pit type shells common to European and Asian almonds. At nut maturity, more than 70 percent of shells of the principle California almond variety 'Nonpareil' can be split, predisposing them to damage by Lepidopterous insects and infection by aflatoxin producing *Aspergillus* strains. The use of a mechanical shaker to harvest nuts increased the proportion of nuts with split-shells by 40 percent when compared to hand harvest. All shell-splitting occurred at the ventral suture with approximately 80 percent of the splits occurring at the site of the degenerating funiculus leading to the aborted or secondary ovule. Remaining splits occurred near the site of the funiculus feeding the viable ovule, and only rarely at the suture line. Since the abortion of one of the two ovules in the almond ovary is typically initiated at or shortly following flower development, the final site of shell-splitting appears to be predetermined early in fruit development. Measurements of the strength of the inner endocarp wall at 50 days after flowering showed distinct weaknesses in the areas of the developing funiculi. Similarly, damage to the developing kernel at 60 days after flowering by the Hemipterous insect pest leaffooted bug (*Leptoglossus clypealis*) occurred along the ventral suture, with 80 percent of the damage located at the point of attachment of the secondary funiculus . An almond shell-split model based on an altered development of the abortive funiculus has been useful in assessing cultural management methods to minimize shell-splits in the field. This model has also allowed the development of efficient selection methods for breeding resistance to shell-splits and associated insect damage.

PANEL DISCUSSION: Enhancing Crop Resistance by Conventional Breeding

Panel Chair: Don White

Panel Members: Robert Brown, Tom Gradziel, Bao Zhu Guo, C. Corley Holbrook and Tom Isakeit.

Summary of Presentations: The moderator of this session was W. Paul Williams, USDA-ARS. There were seven presentations covering various aspects in the use of conventional approaches for developing improved crop resistance in peanut, maize and almonds. Good progress is being made toward identifying and using resistance for control of *Aspergillus* infection and the production of aflatoxin. Results presented at this session and sessions in previous years clearly point to the fact that conventional breeding must play a major part in reducing aflatoxin contamination in corn, peanut and almond. Various levels of success has been achieved with different commodities, in part, because of the traits growers are willing to accept in a variety that produces low levels of aflatoxin and the agronomic characteristics of known sources of resistance.

Corn: Don White, Robert Brown, Bao Zhu Guo and Tom Isakeit presented summaries of research done at various locations with corn (see abstracts of presentations and posters). The corn research can generally be classified into three major areas. With the first research area both elite and exotic germplasm is being evaluated to identify sources of resistance. At present there are no elite inbred lines with resistance to aflatoxin production that can be used directly in commercial hybrids that will vastly improve the agronomic performance and resistance of the commercial hybrid. Several new sources of resistance have been identified and were reported during the session. The second area of research involves identification of mechanisms associated with resistance. This has resulted in basic understanding of a number of different mechanisms including chemical composition of kernel wax, several different proteins including a trypsin inhibitor, numerous stress-related proteins, maysin which is active against corn ear worm, etc. The third area of research is identification and use of molecular markers associated with resistance to cross resistance from various sources into agronomically acceptable inbred lines. It is very apparent that the success in controlling aflatoxin production in corn is dependent upon all three of these research areas. We now have a number of sources of resistance and in some cases understand the mechanism of resistance. We are now keenly aware of the fact that no one source or mechanism of resistance will completely solve aflatoxin contamination problems. Because of the complexity of the resistance it is absolutely imperative to use molecular markers to cross resistance into commercially acceptable germplasm and to use markers to recover the favorable agronomic characteristics of the recurrent parent.

Peanuts. With peanut research, methods for identification of resistance have been developed and 14 sources of resistance have been identified. The research has also identified significant reduction in aflatoxin production in peanut genotypes that have high levels of drought tolerance. The drought tolerant genotypes have been used in an applied breeding program to recover resistant cultivars. In addition to resistance to aflatoxin production, the peanut breeding program is working to obtain high yield, acceptable grade, and resistance to tomato spotted wilt virus which is a significant disease problem of peanuts. Resistant genotypes with favorable agronomic characteristics are being developed. The progress in peanut seems better than the progress with corn partially because resistance has been found in some genotypes with acceptable agronomic characters.

Tree nuts (almond). Perhaps the most successful of all the breeding programs is tree nuts. With several different tree nuts it has been well documented that insect damage to developing fruit is highly associated with aflatoxin contamination. Therefore, control of insect damage will control the problem. With almond it has been demonstrated that the fungal resistance within the almond mesocarp, endocarp, and seed tissue also exist. With almond, resistance and cultural management methods to minimize shell-splits in the field will likely result in acceptable control of aflatoxin production.

Summary of Panel Discussion: Panel discussion was brief because discussion time was reduced. One question that arose was AHow much yield are growers willing to give up in order to get control of aflatoxin?@ With almond it was indicated that growers are not giving up much yield but will accept differences in tree architecture, etc. Peanuts growers are not likely to give up yield and fortunately resistance exists in some genetic backgrounds with good agronomic characteristics. With corn, members of the Texas Corn Growers Association indicated that farmers would not be willing to give up yield and the panel agreed that the farmers would require that varieties with resistance to aflatoxin production must have very favorable agronomic characteristics such as standability, resistance to other diseases, and yield. Given the rapid changing nature of corn hybrids and the large number of corn hybrids on the market today, it is likely that breeding for disease resistance for corn will be the most complicated. It seems, however, that corn has a major advantage in that molecular markers are in use and will allow for recovery of agronomic characteristics.

There also was a discussion on the use of transgenes for the control of aflatoxin production in corn. Don White indicated that work at the University of Illinois has had limited success. Transgenes that have resistance have been produced but the level of resistance is not as great as that of some naturally occurring inbreds. No one resistance mechanism that will provide complete control of aflatoxin production is known. There also was a discussion on phytoalexins related to resistance. No one on the panel or the audience knew if phytoalexins could be involved in resistance.

In general, there was agreement that good progress is being made.

AFLATOXIN RESISTANCE OF SUBTROPICAL/TROPICAL QUALITY PROTEIN MAIZE HYBRIDS IN TEXAS

S. Bhatnagar, F. J. Betrán and D. Transue. Soil and Crop Sciences Department, Texas A&M University, College Station, TX

The nutritional quality of maize can be dramatically improved by selecting for mutations in the *Opaque-2* (o2) gene. The reduction of α-zeins by the *opaque-2* allele results in a proportional increase in the relative amounts of lysine and tryptophan. Unfortunately, the improved nutritional value of o2 maize is associated with grain yield reduction, greater insect and disease susceptibility and undesirable kernel characteristics. To correct these deficiencies, o2 modifier genes were introduced by selecting for a hard, vitreous endosperm in o2/o2 populations. This combination of o2 and genetic modifiers was designated as Quality Protein Maize (QPM). CIMMYT has developed QPM adapted to a wide range of subtropical and tropical environments while sustaining high yields and improved agronomic performance. Pre-harvest aflatoxin contamination (AF), a potent carcinogen produced by the fungus *Aspergillus flavus*, is a major problem for corn growers in Texas. The response of elite QPM hybrids to aflatoxin resistance under Texas conditions is unknown and conditions their adoption. Our objective was to evaluate subtropical/tropical QPM hybrids for AF resistance in Texas conditions.

White and yellow CIMMYT QPM hybrids were evaluated for response to AF at two Texas locations, one subtropical (Weslaco - WE) and other more temperate (College Station - CS) during year 2000. QPM hybrids and non-QPM commercial hybrids were planted using an alpha-lattice experimental design with 4 replications. Traits measured were AF in parts per billion (ppb), husk cover (HC) in a visual rating of 1 (good) to 5 (bad), and ear rot (ER) in a visual score of 1 (no ear rot) to 5 (high ER). Fifteen plants per plot were inoculated with 3 ml conidial suspension per ear containing 10^7 conidia per ml of *A. flavus* (strain NRRL 3357) 6 – 10 days after midsilking using the silk channel inoculation technique. Drought and heat stress was induced by late planting and limited irrigation. Quantification of AF was determined using the Vicam Aflatest™.

Significant differences were observed at both locations. Average AF for white hybrids at CS was 168.2 ppb (range: 25.5 to 655.0 ppb) and 541.4 ppb at WE (range: 97.75 to 1462.5 ppb). Average AF for yellow hybrids was 141.46 ppb at CS (range: 24.5 to 467.5 ppb) and 729.13 ppb at WE (range: 110.5 to 2753.1 ppb). Greater means and variation for AF were observed at WE than at CS. HC and ER showed positive correlation with AF at both locations. AF for yellow QPM hybrids at WE was 512.3 ppb vs. 1454.4 ppb for the checks. AF for white QPM hybrids at WE was 548.72 ppb vs. 933.35 for the checks. White QPM hybrids [CML144 x CML159] x CML176 (25.5 ppb at CS and 98.7 ppb at WE) and CML186 x CML142 (66.5 ppb at CS and 136.6 ppb at WE), and yellow QPM hybrids [CML161 x G26Qc18MH134] x DO940Y (59.7 ppb at CS and 110.4 ppb at WE) and CML161 x CML170 (63.5 ppb at CS and 136.2 ppb at WE) were the less susceptible hybrids to AF. White and yellow QPM hybrids had better husk cover, less ear rot, and were more resistant to aflatoxin as compared to non-QPM checks at both locations. QPM germplasm of subtropical background appears to be a promising source of AF resistant factors.

AFLATOXIN CONTAMINATION OF EARLY, INTERMEDIATE, AND LATE MATURING MAIZE HYBRIDS

Javier Betrán[1] and Thomas Isakeit[2]. [1]Department of Soil and Crop Sciences; [2]Department of Plant Pathology, Texas A&M University, College Station, TX

Preharvest aflatoxin (AF) contamination is one of the main limitations for corn production in large areas of Texas. At present, there are no AF-resistant commercial hybrids for these areas, which experience hot, dry conditions later in the growing season. One possible approach to reduce AF contamination is to utilize short season corn varieties that escape much of the heat and drought stress. The objective of this study was to compare the aflatoxin contamination of early, intermediate and full season commercial hybrids. A total of 25 commercial hybrids representing field and food corn and a gradient of different maturities, including full-season (>115 days to relative maturity, DRM), intermediate (100-110 DRM) and early (< 95 DRM) hybrids and checks were evaluated in two locations in Texas, College Station (CS) and Weslaco (WE), with and without inoculation with *Aspergillus flavus*. The early maturity hybrids used in this study are commonly planted in the Midwestern USA corn belt. An alpha-lattice experimental design was used, with 4 replications for the aflatoxin trials and with 2 replications for the yield trials. Plots were 6.4 m long and 0.75 m wide. The silk channel technique was used to inoculate ears 6-10 days after midsilk. Drought and heat stresses were induced by late planting and limited irrigation. Flowering dates were recorded as days from planting to 50% of plants in the plot showing silks. Husk cover was rated on a 1-5 scale (1=good). At harvest, infected ears were husked, rated for insect injury and visible fungal colonization, dried, shelled, and bulked. The whole kernel sample was ground with a mill and evaluated for AF using the Vicam Aflatest™ system. There were significant differences among the hybrids for AF, silking date, husk cover and grain yield. AF were relatively high in both locations. Average AF at College Station was 926 ppb (range from 176 to 2033 ppb) and at Weslaco 1742 ppb (range from 282 to 3550ppb). Full-season hybrids had lower AF than intermediate and early hybrids. The correlation between flowering date and AF was significantly negative in both locations (-0.59 at CS and -0.58 at WE). Average silking date at CS was 82.9 days (range from 75.6 to 89.1) and at Weslaco 66.7 days (range from 61.3 to 72.0). Husk cover was better in full-season hybrids than in other hybrids. The correlation between poor husk cover ratings and AF was significantly positive in both locations (0.77 at CS and 0.76 at WE). The average husk cover at College Station was 2.4 (range from 1 to 3.8) and 3 at Weslaco (range from 1.3 to 4.3). The grain yield average at College Station was 3.5 t/ha (range from 0.7 to 7.2), which is relatively low because a hail storm caused extensive damage a few days before flowering, and 6.7 t/ha at Weslaco (range from 4.4 to 10). There was a negative correlation between grain yield and AF in both locations. The full-season hybrids had higher yields than hybrids that matured earlier. The data indicates that early-maturing varieties used in the Midwest are not suitable for planting in Texas as an approach to manage AF. If early maturation is a viable approach, then such varieties should be agronomically adapted for Texas conditions.

AFLATOXIN ACCUMULATION IN RESISTANT AND SUSCEPTIBLE CORN GENOTYPES INOCULATED WITH *Aspergillus flavus* USING WOUNDING AND NON-WOUNDING TECHNIQUES

G. L. Windham and W. P. Williams. USDA-ARS, Corn Host Plant Resistance Research Unit, Mississippi State, MS

The most desirable method of aflatoxin control in corn is host plant resistance. Progress has been made in identifying sources of resistance. It was suggested that internal (e.g., anti-fungal proteins) and external (e.g., surface waxes, thickened pericarp) characteristics of kernels account for the resistance of certain genotypes. It is not known which factor is the most important in protecting corn genotypes from invasion by *A. flavus* and aflatoxin formation. This study was to determine if aflatoxin levels increased in resistant and susceptible genotypes if external barriers were bypassed using wounding inoculation techniques.

Corn genotypes included an *A. flavus* resistant population (Gtmas:gk), an *A. flavus* resistant hybrid (Mp313E x Mp420), and an *A. flavus* susceptible hybrid (Ab24E x SC229). Two popcorn populations [MoSQA-C10 and WPC(H23)-C0] with thickened pericarps were also included in the study. The inoculation techniques included the silk channel technique (non-wounding), the side-needle technique (light to moderate wounding), and the knife technique (heavy wounding). Ears were inoculated with *A. flavus* (isolate 3357) 7 days after midsilk using the silk channel and side-needle techniques or 21 days after midsilk using the knife technique. Ears were harvested ca. 63 days after midsilk and aflatoxin contamination was determined using the Vicam Aflatest.

In 1998, environmental conditions were conducive for the production of high levels of aflatoxin in the corn kernels. In the resistant population, Gtmas:gk, and in the susceptible hybrid, Ab24E x SC229, aflatoxin levels were high regardless of the inoculation technique. In the popcorn genotypes, MoSQA-C10 and WPC(H23)-C0, aflatoxin levels were significantly higher in plots inoculated with the side-needle technique compared to the silk channel technique. The lowest amount of aflatoxin was found in the resistant hybrid, Mp313E x Mp420. Plants of this resistant hybrid inoculated with the side-needle technique had significantly more aflatoxin compared to plants inoculated with the knife technique. In 1999, very few differences were observed between inoculation techniques. The side-needle technique had higher levels of aflatoxin than the knife technique in the popcorn population, MoSQA-C10, and in the susceptible hybrid, Ab24E x SC229. Whereas in the popcorn population, WPC(H23)-C0, the aflatoxin levels were higher in the knife inoculated plots than in the side-needle inoculated plots. In the resistant genotypes, Gtmas:gk and Mp313E x Mp420, no differences in aflatoxin levels were observed between inoculation techniques. The amount of wounding caused by an inoculation technique had little effect on aflatoxin contamination of kernels. Some inoculation techniques may be superior to others under certain conditions, but the amount of wounding does not appear to be that important. Therefore, external resistance factors such as thickened pericarp or surface waxes may play a small role in the prevention of aflatoxin contamination. It appears that internal resistance factors such as anti-fungal proteins may play a greater role in the prevention of aflatoxin contamination in corn kernels on developing ears.

RFLP ASSESSMENT ON HETEROGENEOUS NATURE OF MAIZE POPULATION GT-MAS:GK AND FIELD EVALUATION OF RESISTANCE TO AFLATOXIN PRODUCTION BY *Aspergillus flavus*

A. Butron[1], H. Li[2], B. Z. Guo[1], N. W. Widstrom[3] and R. E. Lynch[1].
[1]USDA-ARS, Crop Protection and Management Research Unit, Tifton, GA; [2]University of Georgia, Coastal Plain Experiment Station, Tifton, GA; [3]USDA-ARS, Crop Genetics and Breeding Research Unit, Tifton, GA

The challenge to breeders/plant pathologists is to identify lines that have resistance to aflatoxin production. Maize population GT-MAS:gk has been identified and released as a germplasm with resistance to aflatoxin contamination. In the present study, we assessed genetic divergence in the GT-MAS:gk population using RFLP DNA markers to survey 11 selfed inbred lines and conducted field evaluations for the dissimilarities in aflatoxin production among these inbred lines in comparison with a sister population, GT-MAS:pw,nf. The 11 selfed inbred lines were assayed for DNA polymorphism using 113 restriction fragment length polymorphism (RFLP) markers in 10 linkage groups covering 1518.2 cM. Considerable variation among the inbreds was detected with RFLP markers of which 42 probe-enzyme combinations gave 102 polymorphic bands. Cluster analysis based on genetic similarities revealed associations and variations among the tested lines. Three polymorphic groups were distinguished by cluster analysis. A two-year field evaluation data showed that aflatoxin concentration among the lines were significantly different in both years ($P< 0.001$). The maturity data were also different. Thus, this study demonstrated that the maize population GT-MAS:gk is heterogeneous and individuals may be different in resistance to *A. flavus* infection and aflatoxin production. Therefore, the most resistant lines should be inbred to increase homogeneity and resistance should be confirmed through progeny testing.

RFLP MARKERS ASSOCIATED WITH SILK ANTIBIOSIS AND PCR-BASED MARKERS FOR MARKER-ASSISTED SELECTION

B. Z. Guo[1], G. Xu[2], Y. G. Cao[2], N. W. Widstrom[3] and R. E. Lynch[1].
[1]USDA-ARS, Crop Protection and Management Research Unit, Tifton, GA; [2] University of Georgia, Coastal Plain Experiment Station, Tifton, GA; [3]USDA-ARS, Crop Genetics and Breeding Research Unit, Tifton, GA

Maysin, a C-glycosylflavone in maize silk, has insecticidal activity against corn earworm (*Helicoverpa zea* Boddie) larvae. Sweet corn (*Zea mays* L.) is a vulnerable crop to ear-feeding insects and requires pesticide protection from ear damage. This study was conducted to identify maize chromosome regions associated with silk maysin concentration and eventually to transfer and develop high silk maysin sweet corn lines with marker-assisted selection (MAS). Using F_2 population derived from dent corn (high maysin) and sweet corn (low maysin sh2, we detected two major quantitative trait loci (QTL), *npi286* (flanking to *p1*) on chromosome 1S and *a1* on chromosome 3L. Locus *a1* has a recessive gene action for high maysin with the presence of functional *p1* allele. Markers *umc66a* (near *c2*) and *umc105a* on chromosome 9S also detected in this analysis with minor contribution. A multiple-locus model, which included *npi286*, *a1*, *csu3* (Bin 1.05), *umc245* (Bin 7.05), *agrr21* (Bin 8.09), *umc105a*, and the epistatic interactions *npi286* x *a1*, *a1* x *agrr21*, *csu3* x *umc245*, and *umc105a* x *umc245,* accounted for 76.3% of the total silk maysin variance. Tester crosses showed that, at the *a1* locus, dent corn has functional *A1* alleles and sh2 sweet corn has homozygous recessive *a1* alleles. Individuals of BC1 were examined with MAS and plants with *p1* allele from dent and homozygous *a1* alleles from sweet corn had consistent high silk maysin. Marker-assisted selection seems to be a suitable method to transfer silk maysin in these lines. For the practical application in marker-assisted selection, we have generated PCR-based markers from RFLP markers.

IDENTIFICATION OF PUTATIVE GENES RELATING TO DROUGHT STRESS IN MAIZE BY DIFFERENTIAL DISPLAY OF mRNA

Y. G. Cao[1], B. Z. Guo[2], R. D. Lee[1] and R. E. Lynch[2]. [1]University of Georgia, Coastal Plain Experiment Station, Tifton, GA; [2]USDA-ARS, Crop Protection and Management Research Unit, Tifton, GA

Plants respond to many types of environmental stresses. Among these, the drought stress is the most serious problem that limits plant growth and crop production in agriculture. When plants are stressed by environmental factors, a series of physiological and biochemical changes occur based on the gene expression. Drought stress and *Aspergillus* infection also exacerbate the aflatoxin contamination in corn. Differential display technology of mRNA is a powerful tool for identifying and cloning differentially expressed genes. Our research goal is to identify and clone the genes regulated by drought stress using this technique and characterize these genes in relationship with drought tolerance in corn and aflatoxin formation. In order to clone the genes that relate to drought stress in maize, the differential display RT-PCR technique has been used by generating a differential expression profiles between plants with drought stress vs without drought stress. Leaf tissues were collected at 0, 2 and 4 days after induction of drought stress. Total RNA was extracted and subjected to differential display using specific and random primers. The differentially expressed fragments, which were up- or down-regulated by drought stress, were excised from the gels, re-amplified by PCR, and verified by reverse Northern blot. Sixty-eight cDNA fragments were obtained. Fourteen cDNA fragments were sequenced and analyzed using BLAST in the GenBank. Among these, 2 cDNA fragments have homology with known sequences. Further Studies are needed to characterize these cloned fragments in relationship with drought tolerance and aflatoxin formation.

CLONING AND SEQUENCING OF A FULL-LENGTH cDNA ENCODING FOR PHOSPHOLIPASE D IN PEANUT

B. Z. Guo[1], G. Xu[2], C. C. Holbrook[3] and R. E. Lynch[1]. [1]USDA-ARS, Crop Protection and Management Research Unit, Tifton, GA; [2]University of Georgia, Coastal Plain Experiment Station, Tifton, GA; [3]USDA-ARS, Crop Genetics and Breeding Research Unit, Tifton, GA

Preharvest aflatoxin contamination has been identified by the peanut industry as the most serious challenge facing the industry worldwide. Drought stress is the most important environmental factor exacerbating *Aspergillus* infection and aflatoxin contamination in peanut. Development of resistant peanut cultivars would represent a major advance for the U.S. peanut industry. In this study, we identify a novel *PLD* gene, encoding a putative phospholipase D, a main enzyme responsible for the drought-induced degradation of membrane phospholipids in plants. The completed cDNA sequence was achieved by using the consensus-degenerate hybrid oligonucleotide primer strategy. We have used the sequence information encoded by the cloned fragments to amplify both the 5' and 3' ends of this gene to obtain a full length clone. The deduced amino acid sequence shows high identity with known *PLD* genes, having similar conserved features, such as two H*X*K*XXXX*D motifs. Further study is needed to genetically and physiologically characterize the PLD in peanut and to gain a better understanding of its function and relationship with drought-tolerance.

IDENTIFICATION OF THE DROUGHT-INDUCIBLE GENES IN PEANUT BY mRNA DIFFERENTIAL DISPLAY

G. Xu[1], B. Z. Guo[2] and R. E. Lynch[2]. [1]University of Georgia Coastal Plain Experiment Station, Tifton, GA; [2]USDA-ARS, Crop Protection and Management Research Unit, Tifton, GA

Drought stress severely affects yield and predisposes peanut to preharvest aflatoxin contamination. Breeding for drought tolerant lines is one of the strategies in developing aflatoxin-tolerant peanut lines. Hence, understanding the mechanism of drought tolerance and developing molecular markers are needed for screening and characterizing peanut germplasm for drought tolerance in relation with aflatoxin contamination. There is no report on genetic mapping of molecular markers linked to drought tolerance in peanut, which is very difficult and time consuming. The alternative approach is to monitor changes in gene expression as affected by induced water stress and to identify differentially expressed transcripts. In order to differentiate gene expression in the drought-susceptible and drought-tolerant peanut genotypes, the differential display RT-PCR has been applied to investigate gene expression pattern in response to induced drought stress. One poly(A)-anchored oligonucleotides (H-T11G) and 10 arbitrary primers are used. Various number of bands observed with the combination of primers, 20-40 bands, size ranging from 150 to 500bp. Polymorphic mRNA transcripts have been identified. Some cDNA fragments, up- or down-regulated by induced drought stress, have been cloned and sequenced for BLAST analysis. Eight polymorphic bands have been cloned. One clone sequence has high homology with strawberry 40S ribosomal protein s12 mRNA and the full-length cDNA has been cloned and sequenced as a putative ribosomal mRNA in peanut.

PRE-SCREENING INOCULATED CORN EARS FOR AFLATOXIN USING BRIGHT GREENISH-YELLOW FLUORESCENCE

Steven H. Moore, Dean Lee Research Station, Louisiana State University Agricultural Center, Alexandria, LA.

Measurement of aflatoxin produced by *Aspergillus flavus* Link ex Fr. in corn (*Zea mays* L.) is a relatively tedious and expensive procedure and limits the number of lines that can be tested. The objective of this research was to determine if bright greenish-yellow fluorescence (BGYF) of coarsely cracked kernels could be used effectively to pre-screen ears in segregating populations and thus reduce the number of lines requiring laboratory analyses. A little more than 200 ears from two segregating populations produced in 20 different rows were inoculated with A. *flavus* shortly after anthesis. Following harvest, ears were rated for fungal growth (0-9 scale), then shelled, coarsely ground and rated for BGYF (0-9 scale), then ground to a fine meal and analyzed for aflatoxin (ng g^{-1}). The correlation coefficient between fungal growth and BGYF was 0.55, between fungal growth and aflatoxin was 0.55, and between BGYF and aflatoxin was 0.51 ($p=0.0001$). In most rows, the ear with the lowest BGYF had the lowest aflatoxin. In all rows with an ear rated for BGYF at 2 or less, the ear with the lowest aflatoxin also had a BGYF rating of 2 or less. If a BGYF rating of 2 was used for pre-screening, then 83% of the aflatoxin measurements would have been avoided. A BGYF rating of 2 seems highly useful as a pre-screening tool, should reasonably identify ears with the lowest aflatoxin in segregating populations and could feasibly reduce the number of ears needing tested for aflatoxin in a breeding program by 80% or more.

PLANT TRAITS CONFER *Aspergillus* AND DROUGHT RESISTANCE IN PEANUT VARIETIES

Janjira Puntase[1], Keith Ingram[2], Chuckree Senthong[1] and Corley Holbrook[3]. [1]Chiangmai University, Agronomy Department, Thailand; [2]University of Georgia, Crop and Soil Sciences, Griffin, GA; [3]USDA-ARS, Tifton, GA

Peanuts (*Arachis hypogaea* L.) are susceptible to aflatoxin contamination when pods develop under drought conditions in the field. The objective of this research was to observe root and pod growth of peanut genotypes in response to water stress and *Aspergillus flavus* infection. Four peanut genotypes were grown in 200-L containers with sandy soil. Half of the containers had moisture blocks and thermocouples installed at 5, 25, and 75 cm depth. Soil moisture and temperature were monitored with a CR10X data logger. All containers were inoculated with GFP *A. flavus* cultured on cracked corn medium. Irrigation treatments were well watered (saturated twice a week) and water deficit (saturated twice during one week followed by two week without irrigation). After harvest, pods of each genotype were surface sterilized then cultured on petri dishes with M3S1B medium. Based on mainstem elongation and soil moisture extraction, genoype 329CC appeared to have the greatest drought tolerance, yet stress caused the greatest yield loss for this genotype. Based on pod weight, genotype 511CC was the most drought resistant and also had the lowest *A. flavus* infection in shells. Contrary to expectations, the infection was greater in well-watered than in water-stressed plants, except for Georgia Green, which also had the highest levels of seed infection. High infection of pod (exterior) and shell (interior) were not related to seed infection. Genetic difference in susceptibility to drought stress in peanut was related to difference in *A. flavus* infection.

MOLECULAR MARKER ASSISTED SELECTION FOR RESISTANCE TO AFLATOXIN PRODUCTION IN MAIZE

C. Paul, D. White and T. Rocheford. Department of Crop Sciences,
University of Illinois, Urbana, IL

We are introgressing QTL from resistant sources Tex6 and Mp313E into an elite inbred through marker assisted selection. We identified QTL on chromosome arms 5S and 10L in Tex6 associated with resistance to aflatoxin production. QTL for resistance on chromosome arm 4L in Mp313E were detected by Georgia Davis and colleagues in Mississippi. We are performing line development with marker assisted backcrossing and pyramiding of Tex6 QTL regions on 5S and 10L and the Mp313E 4L QTL region.

Earlier we evaluated 176 Tex6 x B73 $F_{2:3}$, and 100 (Tex6 x B73) x B73 BC_1S_1s in replicated trials in 1996 and 1997, pin board inoculation method was used, symptoms were rated after 45 days of inoculation and infected ears of each family were assayed for aflatoxin B_1 level. This in conjunction with statistical analyses provided information for QTL from Tex6 for marker assisted selection. For the QTL in Mp313E identified in Mississippi, we developed (Mp313E x Fr1064) x Fr1064 BC_1 plants and genotyped these with SSRs on chromosome arm 4L. We selected for the whole 4L arm from Mp313E since there may be more than one QTL present on this chromosome arm. We crossed the selected BC_1 plants to FR1064 to develop BC_2 plants. The BC_2 plants were grown in Hawaii and crossed to FR1064 to develop BC_3 plants.

The BC_3 plants were genotyped and again selected for heterozygous condition for long arm of chromosome 4 from Mp313E. Selected BC_3 plants heterozygous for long arm chromosome 4 segment with Mp313E QTL were selfed. Near-Isogenic lines will be developed in Hawaii to enable confirming the Mp313E QTL in Fr1064 background. Some selected BC_3 plants were crossed to FR1064 to develop BC_4 seed.

Concurrently (Tex6 x B73) x B73 BC_1S_2 plants were genotyped. Those with the Tex6 QTL regions on 5S and 10L present in either homozygous or heterozygous condition from a family with overall lower levels of toxin production were selected. Tex6 x B73 BC_1S_2 plants with the two Tex6 QTL on 5S and 10L were crossed to Mp313E/Fr1064 BC_3 plants with the Mp313E 4L QTL. F_1 seeds will be planted from the cross of heterozygous plants for the Mp313E QTL and heterozygous and homozygous plants for the Tex6 QTL. F_1 plants will be genotyped and selected for presence of heterozygous condition for all three QTLs and these plants self pollinated. The resultant selected F_2 ears of seed will be planted this winter and seedlings genotyped. Ideally we will identify plants homozygous for all three QTLs and these will be selfed. However we may need to select plants that are heterozygous for one or two of the QTL and fix those QTL in the following generation if necessary. This inbred material will be evaluated for resistance to aflatoxin in Summer 2002.

Thus we have used marker assisted selection to pyramid QTL from Tex6 and Mp313E into an FR1064 background. This represents the first time that QTL from very different genetic backgrounds have been pyramided together, and put into an elite background.

14TH ANNUAL AFLATOXIN ELIMINATION WORKSHOP

SESSION 5: CROP RESISTANCE – GENETIC ENGINEERING

Moderator: ***Phil Wakelyn, National Cotton Council.***

DEVELOPMENT OF TRANSGENIC PEANUT WITH ENHANCED RESISTANCE AGAINST PREHARVEST AFLATOXIN CONTAMINATION

Arthur Weissinger[1], Minsheng Wu[1], Yan-Sheng Liu[1], Keith Ingram[2], K Rajasekaran[3] and T.E. Cleveland[3]. [1]North Carolina State University, Raleigh, NC; [2]University of Georgia, Griffin, GA; [3]USDA-ARS Southern Regional Research Laboratory, New Orleans, LA

Transformation of plants with genes encoding various toxic peptides has been proposed as a means of combating fungal pathogens. An interesting approaches involves the use of wholly synthetic Peptidyl Membrane Interactive Molecules (MIM®), small peptides which were developed by Demegen, Inc., some of which closely mimic naturally occurring molecules such as cecropins.

We attempted to make transgenic peanut plants using a gene encoding Peptidyl MIM® "D5C", an alpha-helical peptide (MW 4000) that is highly active against *A. flavus*. Of 128 putative transgenic plant lines recovered, fifteen carried the D5C transgene, but none contained D5C mRNA, nor was the D5C peptide detected by western blots capable of detecting the peptide at a level of 1 ng, or about 0.3% of total soluble protein. Primary transgenic peanut plants yielded significantly fewer pods than control plants. Bioassays for antifungal activity of leaf extracts against A. *flavus* and *Verticillium dahiliae* failed to detect any significant difference between transgenic and control plants. These, and subsequent experimental results indicate that D5C exhibits marked phytotoxicity at levels known to be required to kill A. *flavus*. Further, these results suggest that the phytotoxicity of D5C makes it unsuitable for antifungal applications in peanut. For this reason, further work with D5C has been abandoned.

Results with D5C suggest that all transgenes encoding peptides with antifungal activity should be tested for phytotoxicity before making the substantial investment required to develop transgenic plants. This can be achieved by treatment of explants with purified peptide. Where this cannot be done, the phytotoxic effect of the transgene can be determined by measuring if the gene alters transformation efficiency in e.g., tobacco suspension culture cells. Such cultures are readily transformed by microprojectile bombardment, and can be selected quickly on media containing Kanamycin. Results from our tests with the D5C transgene are clearly consistent with the hypothesis that the gene product is toxic and therefore reduces transformation efficiency in this system.

In other work, we determined whether matrix attachment regions (MARs) flanking the transgene would increase expression in peanut. These DNA sequences have been shown to increase both transient and stable transgene expression, and to stabilize the transgenic phenotype over generations. Embryogenic calli of peanut cv. NCV11 were bombarded with CaMV 35S*uid*A (GUS) with and without flanking MARs derived from the Rb7 tobacco gene. Care was taken to minimize experimental error due to differences in calli. Explants were assayed 48 hours after bombardment with the GUS-Light (Applied Biosystems) system. Current results indicate that the Rb7 MARs are effective in peanut. GUS expression in tissues bombarded with the GUS cassette flanked by Rb7 MARs was up to eight-fold higher than that in tissues bombarded with an identical GUS cassette without MARs. This work will be expanded to measure the effect of MARs on the frequency of stable transformation and long-term phenotypic stability in peanut.

TRANSGENIC PEANUT FOR PREHARVEST AFLATOXIN REDUCTION

Peggy Ozias-Akins[1], Hongyu Yang[1], Evelyn Perry[1], Yoko Akasaka[1], Chen Niu[1], Corley Holbrook[2] and Robert Lynch[2]. [1]Department of Horticulture and [2]USDA-ARS, University of Georgia Coastal Plain Experiment Station, Tifton, GA

The insecticidal crystalline protein from *Bacillus thuringiensis* (*Bt cryIA(c)*) was introduced into peanut several years ago. Field tests were conducted to assess the level of insect resistance and the level of aflatoxin reduction associated with insect resistance. Both lesser cornstalk borer (LCB) infestation and *Aspergillus* inoculation were carried out in these field tests. Leaves from *Bt*-expressing plants showed significantly less insect damage than control plants. In 2000, the percentage of pods showing scarification damage from LCB was not significantly different between transgenic plants (20%) and non-transgenic plants (23.5%). The intensity of scarification was not measured. There was a significant difference for aflatoxin levels between the scarified transgenic and scarified non-transgenic samples, although the number of replicates was small (approx. 5 samples for each class). The experiment was repeated in 2001 with additional replicates. LCB damage and aflatoxin levels will again be quantified. During the course of plant development, all individuals were tested for expression of the *Bt* toxin. Leaf samples taken one month after planting clearly distinguished between transgenic, expressing and non-transgenic, non-expressing individuals. The average leaf expression levels in the two lines tested were 15 and 17 ng/ml of toxin. Protein levels will also be assayed in these samples to determine the amount of toxin per total protein. Leaves from a subsample of expressing plants showed an increase in toxin levels one month after stress. Pods and seeds sampled from the same individuals showed 20-35 ng/ml of toxin, approximately double the concentration observed in leaf samples. Again, final conclusions on toxin concentration must await the determination of total protein content. To determine the efficacy of CryIA(c) compared with other *Bt* toxins, in vitro feeding assays were conducted. Out of eight toxins tested, only one was similar to CryIA(c) in providing a LC_{50} at 0.09 ìg/ml and a LC_{95} at 0.18 ìg/ml.

Bioassays have been completed on a transgenic peanut line segregating for expression of tomato anionic peroxidase. Although a trend was observed for less LCB foliage damage and fewer colonies of *Verticillium dahliae* growth on leaf extract, no significant differences were observed between transgenic and non-transgenic plants. A different type of peroxidase gene, a bacterial chloroperoxidase, was obtained from Kanniah Rajasekaran and Jeff Cary (USDA-SRRC, New Orleans). This gene has been introduced into peanut, and transgenic lines have been recovered. The transgenic lines will be tested for expression, and tissue extracts will be used for bioassays before plants are regenerated.

Mercury resistance can be conferred to shoots by the introduction of the *merA* gene under the control of the *Arabidopsis* actin 2 promoter. Although it may be possible to develop a transformation system based on mercury resistance and shoot regeneration, we have not succeeded in applying mercury selection for the recovery of transgenic somatic embryos. An alternative screenable marker, green fluorescent protein, has resulted in the recovery of transgenic peanut.

ANTIMICROBIAL PEPTIDE TECHNOLOGY TO PREVENT FUNGAL AND BACTERIAL DISEASES OF CROPS

Paul S. Zorner, Demegen Inc., Pittsburgh, Pennsylvania.

Insect injury, fungal and bacterial diseases, aflatoxin contamination and weed pests can cause major reductions in yield, quality and safety of foods and feeds, thus sometimes leading to severe economic losses for the growers. Newly available technologies utilizing antimicrobial peptide (AMP) genes that can be "stacked" along with anti-insect and herbicide-tolerance traits in transgenic crops could help alleviate these economic losses and improve food quality. AMP's are small, amphipathic proteins ranging in size from 15- 45 amino acids and have shown promise to control important plant pathogens in both *in vitro* and *in vivo* studies. Many AMP's being explored today are derived from natural homologs found in virtually all higher organisms. Demegen AMP's are composed of L-amino acids but further "designed" from natural homologs to improve activity and *in vivo* stability. Demegen has designed and tested over 250 peptides on major agricultural pathogens. D4E1 has emerged as Demegen's lead agricultural peptide and has been shown to have significant activity on a variety of bacterial and fungal pathogens such as *Erwinia carotovera*, *Pseudomonas syringae*, and various *Fusarium*, *Verticillium*, *Phytopthera*, *Schlerotinia* and *Pythium* species. Activity on *Aspergillus* species is also evident, but not at levels shown for other pathogens. Development programs are either in place, or being initiated to place D4E1 in a variety of crops such as tobacco, potato, apple, pear, peanut, cotton, grapes, banana and woody ornamentals. Significant hurdles being addressed in these development programs revolve around elevating expression to levels capable of controlling fungal pathogens and developing analytical techniques that allow quantification of peptide *in vivo*. At this point, *in vivo* lab and field studies with transgenic crops have shown good control of bacterial and some fungal pathogens relative to non-transformed controls, but inconsistent control of more tolerant fungal pathogens. Analytical procedures exist to follow DNA and RNA for these peptides. Techniques for direct elucidation of protein have been developed for other Demegen AMP's, but D4E1 has thus far proven more difficult to work with because of its highly amphipathic properties. Work is progressing to resolve these issues, and to look at strategies to bundle these peptides with other important agronomic traits such as insect and herbicide tolerance that are often important factors in a grower's decision to plant conventional or genetically-modified crops.

CONSTRUCTION OF EXPRESSION CASSETTES TO CONFER RESISTANCE TO *Aspergillus flavus* IN COTTON

Caryl A. Chlan[1], Jeffrey Cary[2], Kanniah Rajasekaran[2] and Thomas E. Cleveland[2]. [1]The University of Louisiana, Lafayette, LA; [2] USDA-ARS, Southern Regional Research Center, New Orleans, LA

We have been working to develop cotton that is resistant to the fungal pathogen *Aspergillus flavus* using a genetic engineering approach. Success of this project depends upon the identification of appropriate regulatory elements as well as structural genes that can be linked to confer a new pathogen resistant phenotype to cotton. Based on our previous studies, we have identified several regulatory elements (promoters) that will be useful tools in this approach. These promoter components include native cotton promoter elements derived from a cottonseed storage protein vicilin promoter and the cotton chitinase gene promoter, as well as heterologous promoter elements that are constitutively expressed such as the ubiquitin and Cauliflower Mosaic Virus promoters.

A variety of candidate structural genes whose products may confer resistance to A. flavus have been identified. These include a haloperoxidase gene, a lytic peptide coding sequence, the corn trypsin inhibitor gene, and a cotton chitinase gene. Last year we presented detailed studies characterizing natural cotton defense gene, a neutral cotton chitinase. We also continued our studies in transgenic plants to determine expression levels and efficacy of a variety of promoter/structural gene combinations against different pathogens.

This year, we have focused on generating different promoter/structural gene combinations in the final phase of developing cotton with resistance to *A. flavus*. Our choices of promoter and structural genes has been influenced by several parameters including levels of expression, specificity of expression, and the efficacy of the structural gene product in inhibiting pathogen growth. Additionally, we want to be sure that we have proprietary rights to all components of our constructs. In the past, several transgenic lines have not been able to be release due to patent right infringement suits, and components that were previously considered to be common domain have retroactively become protected by patents.

To regulate the expression of anti-fungal gene expression, we are focusing on combinations of two derivative of the cottonseed vicilin promoter. We are also working to target the protein encoded by the structural gene using the N-terminal targeting sequence of the native cotton vicilin. These regulatory regions are being used in combinations with a previously characterized haloperoxidase gene, a small lytic peptide gene, and the endogenous neutral cotton chitinase gene. Construction of the regulatory region/structural gene cassettes is well under way. Once these constructs are complete, they will be cloned into two different binary vector systems for study: pBI 121 and a vector supplied by Dow. The binary vector pBI 121 is commonly used, has been used in the majority of our earlier studies, but has some potential problems concerning patent issues. In contrast, the binary vector supplied by Dow was specifically constructed to eliminate these potential problems. Once these constructs are complete, we will immediately begin to study the expression and characterize the resistance phenotype conferred by these novel cassettes to transgenic plants.

ANTIMICROBIAL ASSAYS WITH TRANSGENIC COTTONS

Kanniah Rajasekaran, Jeffrey W. Cary, Tom J. Jacks, and Thomas E. Cleveland. USDA-ARS, Southern Regional Research Center, New Orleans, LA

Several independent transformed lines of cotton expressing the antifungal genes coding for chloroperoxidase (*cpo-p*) or the synthetic peptide, D4E1, have been identified through biochemical and molecular assays. *In vitro* results using crude leaf extracts from transformed cotton plants (R_0 and R_1) indicated significant control of *Verticillium dahliae*, a pathogen very sensitive to these two antifungal proteins. In anti-*A. flavus* assays, we observed reduced number of colonies although the results were not highly significant compared to controls. This is possibly due to either reduced level of gene expression or rapid degradation of antifungal proteins in ground extracts by plant proteases. For example, we observed rapid degradation of D4E1 added to cotton leaf extracts within 30 minutes of incubation. *In situ* assays using immature cottonseeds inoculated with Green Fluorescent Protein-expressing *A. flavus* strain showed that the transgenic plants are capable of delaying and reducing the fungal advance in both seed coat and cotyledons. We are currently planning to utilize seed coat- or seed-specific promoters to improve the expression in cottonseed. Availability of suitable foliar pathogens for testing transgenic cotton plants is being explored for evaluating *in planta* disease resistance. In addition, we are increasing seed from R_1 progeny to assay for seedling diseases caused by any one of the following pathogens: *Pythium, Fusarium, Rhizoctonia solani* or *Theilaviopsis basicola*.

In addition to establishing the disease-resistant phenotype of the transformed cotton plants and their progeny, we continue to address the following important aspects of this project: 1) mode of action of CPO-P - Enzyme kinetic analyses have indicated that peroxidation by hydrogen peroxide of alkyl acids, including acetate, was not the basis for the antifungal activity in transgenic plants expressing the CPO-P. Other possibilities such as the enzyme's hydrolase or esterase activities are now being explored. 2) We are continuing to work with our industry partners to detect and quantify D4E1 by MALDITOF Mass Spectroscopy or developing antibodies specific to the peptide for measuring and evaluating the peptide in transgenic plants. Quantifying small peptide molecules is very difficult without the availability of suitable antibodies. These synthetic peptides have a highly positive charge, tend to aggregate and as a result do not migrate into the gel properly making it difficult to perform Western Blots.

In collaboration with our partners (Mycogen/Dow AgroSciences/Demegen) we have also initiated several new experiments with new constructs, free of proprietary concerns, to develop insect and disease–resistant cottons. Such transgenic plants with stacked genes will provide an additional level of protection from *A. flavus* by reducing pink bollworm damage in cotton bolls in addition to increasing the commercial viability of the product.

PROGRESS TOWARD DETEMINING IF ALPHA-AMYLASE INHIBITORS CAN REDUCE AFLATOXIN CONTAMINATION IN MAIZE

Charles P. Woloshuk, Gyung-Hye Huh and Ahmad Fakhoury.
Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN

Aspergillus flavus is a fungal pathogen of maize causing an important ear rot disease when plants are exposed to drought and heat stress. Our previous research has suggested that α-amylase of *A. flavus* may facilitate the colonization of maize kernels and promote aflatoxin production in the endosperm of infected maize kernels. We also reported that the 36 kDa α-amylase inhibitor purified from *Lablab purpureus* (AILP) inhibits α-amylase from a number of fungi but not those from animal and plant sources. The protein also inhibited conidial germination and hyphal growth of *A. flavus*. Partial peptide sequence of the AILP indicated that AILP is similar to lectin members of a lectin-arcelin- α-amylase inhibitor family described in common bean and shown to be a component of plant resistance to insect pests. AILP also agglutinated papain-treated red blood cells from human and rabbit. These data indicated that AILP represents a novel variant in the lectin-arcelin-α-amylase inhibitor family, having both lectin-like and α-amylase inhibitory activity.

The expression of AILP in maize may result in resistance to *A. flavus* and reduce aflatoxin contamination. To test this hypothesis, we are cloning the gene (*Lai*) encoding AILP and we will express *Lai* in *E. coli* and maize. Toward this goal, we designed degenerate primers based on the peptide sequence of AILP and amplified a part of the gene from genomic DNA of *L. purpureus*. Sequence analysis of the amplified fragment predicts 82 amino acid residues of AILP. Southern blots of genomic DNA from *L. purpureus* probed with the PCR product indicated that *Lai* is a member of a multigenic family containing at least nine genes. We have cloned the upstream and downstream regions of the *Lai* genes by genomic walker PCR. From the DNA sequence analysis of these regions we designed PCR primers to obtain a complete coding region of the *Lai* genes.

GENETIC ENGINEERING AND BREEDING OF WALNUTS FOR CONTROL OF AFLATOXIN

Abhaya M. Dandekar[1], Gale McGranahan[1], Patrick Vail[2], Russell Molyneux[3], Noreen Mahoney[3], Charles Leslie[1], Sandie Uratsu[1], Ryann Muir[1] and Steven Tebbets[2]. [1]Department of Pomology, University of California, Davis CA; [2]Horticultural Crops Research Laboratory, Fresno, CA; [3]UDSA-ARS, Western Regional Research Center, Albany, CA

The tree nut crops in California, including walnuts, represent a 1.5 billion dollar industry, a significant amount of which is exported and thus threatened by additional regulations regarding aflatoxin contamination. Our strategy for reducing aflatoxin contamination has three goals; 1) developing insect resistant plants by reducing infection opportunities through insect damaged tissues, 2) reducing the ability of tissues to support *Aspergillus .flavus* growth and or toxin production and 3) identifying the genes encoding the component(s) in the seed coat of the walnut variety Tulare that cause inhibition of aflatoxin production by *A.flavus*. The experimental strategy to achieve these three goals involves gene testing, breeding and genomics based gene discovery approaches.

For engineering resistance to insect pests we have focused on the *cry*IAc gene from *Bacillus thuringiensis*. We are continuing analysis of tissue obtained from field grown material and continuing work on the comparison of the expression of *cry*IAc using two promoters; CaMV35S and Ubi3 (from potato). In addition to leaf tissues, we have begun to analyze some. Currently we have transgenic walnuts at two locations in California at location A in Northern California we have 128 trees and at location B in the Central Valley we have 111 trees. The plants are doing well and are over 7 feet tall in most cases. Several of the transformed lines have flowered and the nuts harvested. Feeding studies are currently underway with nuts. The feeding analysis thus far has revealed that both constructs, CaMV and Ubi, gave significant results compared to control untransformed walnut tissues. The *cry*IAc gene regulated by the CaMV35S promoter in transformed walnut leaves gave consistently higher mortality and delayed larval development compared to the same gene regulated by the Ubi3 promoter. Activity was higher in young leaves than in mature leaves for both promoters. In fruit (early season whole nuts), CaMV35S again produced better results than the Ubi3 promoter. Preliminary results with nuts supports results obtained with other transgenic tissues with the 35S giving better results as compared to Ubi.

In addition to insect resistance, engineering resistance to *A.flavus* is an important objective. The strategy is not only to limit the ability of this fungus to grow in walnut tissues but to inhibit its ability to make aflatoxin. After trying antifungal genes from other sources (i.e., chitin binding proteins, SAR8.2 , PGIP and RIP) we turned our attention to some of the endogenous proteins in walnut kernels that may have antifungal activity. One of these proteins was vicillin. Vicillins a 7S oligomeric seed storage protein utilized during germination and shown to have antifungal activity against *Fusarium solani*, *Fusarium oxysporium*, *Phytophtora capsici*, *Neurospora crassa*, *Ustilago maydis* and *Botrytis cinerea*. The vicillins have also been shown to display anti-insect activityagainst stored product pest (*Callosobruchus maculates*). A possible mode of action is strong binding with chitin containing structures found in fungal cell walls and midgut of insects. Two vectors were tested one expressing the walnut vicillin as a full length cDNA

(pDU99.3906) and the other construct that expresses the mature protein (pDU99.3841). Both expression cassettes are regulated by the CaMV35S promoter. Tobacco was transformed with these two vectors to evaluate inhibition of aflatoxin and antifungal activity. Although all of the transgenic tobacco lines still remain to be tested no significant inhibition of aflatoxin has yet been reproductively observed as compared to control untransformed tobacco.

Perhaps the most exciting development is identification of constituents in the pellicle of the walnut cultivar Tulare (Tulare Factor) that inhibit aflatoxin production by *A.flavus*. Studies conducted at the USDA facility at Albany (Mahoney et al., unpublished) have shown the presence of at least two sources of natural resistance to *A.flavus* growth and toxin production in walnut kernels. The most active component appears to be the 'Tulare Factor'present in the seed coat of the walnut variety Tulare and results in the complete inhibition of aflatoxin production. A second and more general component has an antifungal property that limits *A.flavus* growth and toxin production in walnut kernels. Mahoney and Molyneux at the USDA Albany laboratory are characterizing the chemical nature of the Tulare factor and we at the Pomology Department are identifying the genes that encode the Tulare Factor. Preliminary results suggest that the Tulare Factor is a hydrolysable tannin. We are currently developing a genomic and proteomic strategy to identify the genes that encode the Tulare Factor. Our strategy for identifying the natural sources of resistance to aflatoxin synthesis in walnut kernels has two components. The first is to evaluate the genetics of the trait and the second is a gene discovery strategy to identify the genetic component. The major outcome of the former objective is the development of molecular markers for screening progeny and breeding selections at the seedling stage to identify those that will bear nuts with increased resistance to *A.flavus* growth and toxin production. The following seedling populations have been generated: Chico x Chico (n=75), Tulare x Tulare (n = 57) Tulare x Chico (n = 37). In terms of aflatoxin production these correspond to: high x high, low x low and high x low. Analysis of the seed, should indicate whether the factor is inherited as a single gene or multiple genes. We are currently making cDNA libraries from the seed coat of kernels obtained from the walnut varieties Tulare and Chandler. Genomics based approaches will be used to identify genes responsible for the active component that encodes the hydrolysable tannin.

PANEL DISCUSSION: Crop Resistance – Genetic Engineering

Panel Chair: Peggy Ozias-Akins

Panel Members: Arthur Weissinger, Paul Zorner, Caryl A. Chlan, Kanniah Rajasekaran, Charles P. Woloshuk and Abhaya M. Dandekar.

Summary of Presentations and Panel Discussion: The moderator of this session was Phil Wakelyn of the National Cotton Council. There were seven presentations covering various aspects in the development of transgenic approaches in peanut, cotton, maize walnuts and other crops. Crop resistance to aflatoxin through genetic engineering remains largely in the development phase though several approaches are showing signs of promise. The types of genes that may show some efficacy against the aflatoxin producer, *Aspergillus*, include those for antimicrobial peptides, peroxidases, and amylase inhibitors; however, expression of only one of these genes in a variety of crops probably will not totally inhibit fungal growth. Incomplete inhibition may be due to several factors including low levels or instability of expression, compartmentalization of expression or its effects, and variable target efficacy. Regulatory elements (e.g. matrix attachment regions (MARs) and seed promoters) that may provide more stable expression levels in target tissues are under investigation. MARs have no specific nucleotide sequence but have AT-rich motifs that bind to the nuclear matrix. The expression of antimicrobial peptides in plants is difficult to monitor and often may be too low to be efficacious at target sites. The question was raised whether a conjugated form could be produced in the plant that would be activated by metabolic activity of the fungus. Another tactic to increase expression of antifungal genes would be chloroplast transformation. Since numerous plastids are present in each plant cell, each can function as a protein synthesis factory. Increased expression also might be possible by arraying several small peptide genes with varying specificities in one construct in order to target a broader spectrum of pathogens. Finally, the possibilities for deployment of transgenic plants expressing antifungal, insecticidal, or anti-aflatoxin genes were discussed. The most successful examples thus far appear to be those crops with a champion in the private sector. Technology transfer to the private sector may include the licensing of intellectual property from academic institutions, but in some cases it may require the private sector to contribute to the patent and regulatory approval process. Perhaps the most effective approach for multiple crop industries would be for a single entity to negotiate a complete transformation intellectual property package that could be licensed as a bundled unit in order to facilitate subsequent commercialization.

AfRTL-1, A RETROTRANSPOSON-LIKE ELEMENT IN THE AFLATOXIN-PRODUCING FUNGUS *Aspergillus flavus*

Patricia Okubara[1], Brian Tibbot[1], Cesaria McAlpin[2] and Sui-Sheng T. Hua[1].
[1]USDA-ARS, Western Regional Research Center, Albany, CA; [2]USDA-ARS, National Center for Agricultural Utilization Research, Peoria, IL

The genomic clone pAF28 was isolated from *Aspergillus flavus* strain NRRL 6541. The clone was used as a molecular hybridization probe in Southern blots to generate restriction fragment length polymorphisms (RFLP) for distinguishing numerous genotypes of *A. flavus* isolated from corn, peanuts, pistachios and almonds. We demonstrate that the genomic insert of pAF28 carries a retrotransposon like element. Retrotransposons are genetic elements that can move to new chromosomal locations via RNA intermediates. Mutations caused by retrotransposon insertions can result in gene activation or in alteration of the expression pattern of genes. Many classes of transposable elements may have been transferred horizontally between species and could contribute to genetic variation in fungi under natural conditions. DNA fragments derived from pAF28 were subcloned into pUC19, transformed into *Escherichia coli* strain JM109. The nucleotide sequence of a 6.3 kb fragment in pAF28 was determined using the ABI Prism BigDye^{TM} Terminator Cycle Sequencing Ready Reaction Sequencer Kit and ABI Prism 310 Genetic Analyzer. Two major overlapping open reading frames (ORFs) of 240 and at least 980 amino acids, respectively, occurred within the 4.5 kb *Sma*I-*Eco*RI fragment of *AfRTL-1*. The first ORF showed identity to polypeptides encoded by *gag* genes of fungal retrotransposons in the gypsy class, including CfT-I from *Cladosporium fulvum*, *skippy* from *Fusarium oxysporum*, and Cgret from *Colletotrichum gloeosporioides*. The putative *gag* ORF of *AfRTL-1* is shorter than those from other sources, due to the presence of a stop codon. However, a GAAAAG beginning at nucleotide 1215 could serve as the site for a -1 frame shift, which would extend the Gag open reading frame into the second ORF. Similar $GA_{4-5}G$ sequences occur in retrotransposable elements from other fungi. A Zn finger RNA binding domain of the consensus $CX_2CX_4HX_4C$, encoded in *gag* regions of retrotransposons, was found at the 5' end of the second ORF. The region of *AfRTL-1* upstream of the Gag ORF contained multiple copies of core enhancer elements and direct repeats. Six *Ty1*/*Neurospora* core enhancer elements (TTCCA) and four *pho80* enhancer elements (TACCA) were located between the *Sph*I and *Sal*I restriction site. Longer elements and direct repeats reported in CfT-I and *skippy* were not observed upstream of *gag* in *AfRTL-1*. However, four new families of direct repeats were identified in this region. Three copies of CTATATAAAA, four copies of ATATTATT, three copies of TTATTTTTA, and three imperfect repeats of the consensus GTATCGACGGCAGTCTAGTGTCGACGGCA were scattered throughout a 850 bp region located downstream of the *Sal*I site. The significance of the direct repeats is not known; however, the shorter repeats might reflect the AT-rich nature of this region. We were unable to identify long terminal repeats (LTR) or duplicated target sequences characteristic of gypsy-class retrotransposons in our partial clone. In view of recent interest in retrotransposons and genetic diversity among *A. flavus* populations, we hope this report stimulates research in elucidating the possible role of such element in *A. flavus*.

EFFECT OF CRYIA(B) ON *Aspergillus* EAR ROT AND AFLATOXIN PRODUCTION IN COMMERCIAL CORN HYBRIDS

L.M. Maupin[1], M.J. Clements[1], S.L. Walker[2] and D.G. White[1].
[1]Department of Crop Sciences, University of Illinois, Urbana, IL;
[2]Monsanto,Haubstadt, IN

The effect of CryIA(b) protein (*Bt*) in corn kernels on susceptibility to *Aspergillus* ear rot and aflatoxin production was studied in two experiments at two locations (southern Indiana and central Illinois) in 2000. In the first experiment, eleven *Bt* corn hybrids and their near-isogenic, non-transgenic counterparts were evaluated. In the second experiment, the same eleven non-transgenic hybrids and an additional hybrid (FRB73xTex6) were pollinated by transgenic or non-transgenic hybrids to produce kernels expressing CryIA(b) and kernels not expressing CryIA(b). Ears were inoculated at the R2 stage with *Aspergillus flavus* using a pinboard inoculator. Aflatoxin was quantified with ELISA.

In experiment one, *Aspergillus* ear rot (P=0.17) and aflatoxin levels (P=0.35) were not significantly different between *Bt* and non-*Bt* hybrids. Hybrid pairs were significantly different (P=0.025) for *Aspergillus* ear rot. The hybrid pair DK537 and DKc53-32 with an average ear rot of 5.81 and another hybrid pair RX508 and RX508YG with an ear rot rating of 5.76 were significantly higher for *Aspergillus* ear rot than all other hybrid pairs.

In experiment two, the source of pollen (*Bt* or non-*Bt*) had no significant effect on *Aspergillus* ear rot (P=0.38) or aflatoxin levels (P=0.57) in grain. Hybrids were significantly different (P=0.0001) for *Aspergillus* ear rot. The same significantly high hybrids in experiment one were also significantly higher than all other hybrid pairs in experiment two. In experiment two, one hybrid B73xTex6 was considerably lower for *Aspergillus* ear rot, and to a limited degree aflatoxin. Tex6 is an unreleased inbred developed at the University of Illinois, which has been identified as resistant to *Aspergillus* ear rot and aflatoxin.

The effect of *Bt* transgenes on reducing *Aspergillus* ear rot and aflatoxin levels in grain may be less important than the influence of background hybrid genetics. Ultimately, the most effective method to reduce *Aspergillus* ear rot and aflatoxin production is through resistant corn hybrids.

AFLATOXIN AND INSECT RESPONSE IN SOUTH TEXAS OF NEAR-ISOGENIC CORN HYBRIDS WITH CRY1AB AND CRY2AB EVENTS

Gary N. Odvody and Charles F. Chilcutt. Texas A&M University
Agricultural Research and Extension Center, Corpus Christi, TX

Monsanto has a regulated (unregistered) *Bt* event (Mon840) with a novel insecticidal protein Cry2Ab that provides more protection against ear feeding lepidopteran insects than Mon810 hybrids expressing Cry1Ab. Our research objectives were to determine *Bt* gene and *Bt* event influence on (1) amount and type of insect injury, (2) pre-harvest aflatoxin content at maturity, (3) agronomic performance, and determine (4) potential relationships among these assessments. Seed of six near-isogenic corn hybrids of B73xH99 was provided by Monsanto for one experiment located in Beeville, TX in 2000. The hybrids with represented Bt events were: B73xH99 (Non*Bt*), Mon810xH99, Mon851xH99, B73xMon840, Mon810xMon840 (stack), Mon851xMon840 (stack). Mon851 is a regulated, proprietary event from Monsanto expressing the Cry1Ab protein. Seed of four near-isogenic corn hybrids was provided by Monsanto for experiments at Beeville and Corpus Christi, TX in 2001. These hybrids included B73xH99 (Non*Bt*), Mon810xH99, B73xMon84006 (a selection out of Mon840), and Mon810xMon84006 (stack). Although the hybrids provided in both years were near-isogenic, the individual lines with transgenes differed from each other in their level of relatedness to the parental lines. The experiment was a randomized complete block design with nine blocks. Within a block each hybrid was randomly assigned to two adjacent 26 ft length rows with a target plant population equivalent to 18-20,000 plants per acre. One row was designated for pre-harvest insect evaluations and ear shank and stalk evaluations at harvest. The other row was for all other at harvest evaluations including insect injury rating of ears and aflatoxin content of grain. Experiments were late-planted to increase potential for drought stress. Primary corn ear insects were corn earworm (CEW, *Helicoverpa zea*) and fall armyworm (FAW, *Spodoptera frugiperda*). FAW can cause some plant and stalk tunneling injury but other stalk borers are generally absent or of negligible importance at most locations in South Texas. Insect pest data included whorl injury rating (Davis et al., 1992), ear insect numbers and larval stage at 18 (2000) and 14-20 (2001) days after silking, and stalk tunneling injury and ear injury rating (Widstrom, 1967) at harvest. Inoculum (autoclaved corn kernels colonized by a high aflatoxin-producing *Aspergillus flavus*, NRRL3357) was distributed between treatment rows at the rate of 1 kg dry seed equivalent/200' when the first hybrids reached mid-silk stage. Ears were harvested after kernel moisture in all hybrids was below 15%. Threshed grain from the nine replicates of each hybrid were pooled into three composite replicates as follows: Reps (1,2,3), (4,5,6), and (7,8,9). All grain from each composite replicate was ground in a Romer mill and a subsample was analyzed for aflatoxin content (ppb) using the Vi-Cam Aflatest P immunoassay system. For most insect parameters evaluated the Non*Bt* hybrid had the greatest insect numbers and injury ratings and the Cry2Ab (Mon840) hybrids had the lowest. Yield of the Non*Bt* hybrid in 2000 was significantly lower than all other hybrids except non-stacked Cry1Ab, Mon851. Yield differences were not significant in 2001 at either location. Aflatoxin content in grain of Cry2Ab hybrids was significantly lower than Non*Bt* and Cry1Ab hybrids in 2000 but

significantly lower than only the Non*Bt* hybrid in 2001. In 2000, the mean number of 4th instar FAW per corn ear at 18 days after silking had a significant positive relationship with ear insect injury rating at harvest and with aflatoxin content. In 2001, the mean number of FAW per corn ear at 14-20 days after silking had a significant positive relationship with ear insect injury rating at harvest and with aflatoxin content. In both years, ear insect injury rating at harvest had a significant positive relationship with aflatoxin content.

Davis, F.M., S.S. Ng, and W. P. Williams. 1992. Miss. Agric. For. Exp. Stn Tech. Bull. 186.

Widstrom, N. 1967. Ear injury rating scale. J. Econ. Entomol. 60: 791-794

14TH ANNUAL AFLATOXIN ELIMINATION WORKSHOP

SESSION 6 POTENTIAL USE OF NATURAL PRODUCTS FOR PREVENTION OF FUNGAL INVASION AND / OR AFLATOXIN BIOSYNTHESIS IN CROPS

Moderator: ***Bob Klein, California Pistachio Commission***

ANTI-AFLATOXIGENIC CONSTITUENTS OF 'TULARE' WALNUT

Russell J. Molyneux[1], Noreen Mahoney[1], Bruce C. Campbell[1], Gale McGranahan[2] and Jim McKenna[2]. [1]USDA-ARS, Western Regional Research Center, Albany, CA; [2]Department of Pomology, University of California, Davis, CA

The current export market for tree nuts is in excess of $1 billion but this market is threatened by the European Community requirement of 2 ppb aflatoxin B_1. Identification of endogenous constituents with the ability to inhibit growth of *Aspergillus flavus* or suppress the biosynthesis of aflatoxins has the potential to prevent contamination of tree nuts prior to harvest and confer resistance to post-harvest contamination.

Walnuts show less propensity for aflatoxin contamination than other tree nuts and considerable differences in susceptibility between cultivars. *In vitro* experiments have shown that the cultivar 'Tulare' has exceptional ability to suppress formation of aflatoxins. Additional studies have demonstrated that this property is independent of growing location or rootstock. Testing of different parts of the nut established that the resistance factor(s) is restricted to the seed coat (pellicle) but is not present in the kernel, and that its activity increases with maturity. Sequential extraction with solvents of increasing polarity showed that the activity was restricted to polar compounds. Analysis of the fractions by gas chromatography- mass spectrometry (GC-MS), permitted the major components of each extract to be characterized and identified specific compounds that may act as aflatoxin resistance factors.

INFLUENCE OF NAPHTALENE-ACETIC ACID ON GROWTH, DIFFERENTIATION AND MYCOTOXIN SYNTHESIS BY *Aspergillus nidulans* AND *A. parasiticus*

Carlos M. Bucio-Villalobos[1], Hugo A. Luna-Olvera[2] and Doralinda Guzmán-de-Peña[3]. [1]Instituto de Ciencias Agrícolas, Universidad de Guanajuato, México; [2]Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León, México; [3]Laboratorio de Micotoxinas, Unidad Irapuato, Centro de Investigación y Estudios Avanzados IPN, México

This study was undertaken to evaluate the effect of plant growth regulators in growth, differentiation and mycotoxin synthesis in *Aspergillus nidulans* and *A. parasiticus*. Conducive proper cultural conditions for mycotoxin synthesis for both organisms and three concentrations of the plant growth regulators were used. Naphthalene-Acetic Acid (NAA) was one out of ten that showed dual effect. Growth, sporulation and sterigmatocystin synthesis were inhibited by 0.5 mM of NAA in *A. nidulans*, while in *A. parasiticus* growth was reduced 32 % and sporulation and aflatoxin synthesis were stimulated. Higher molarity (50 mM) inhibited growth by 75 % and AFB_1 production by 25 %. In contrast, sporulation remained stimulated. These results with NAA suggest that the common regulator point between sporulation and mycotoxin synthesis behave different in *A. nidulans* and *A. parasiticus*. Studies are underway to elucidate the effect of NAA on the transcription of genes involved in sporulation and mycotoxin synthesis.

This project was partially supported by: CONACYT-Mexico, CINVESTAV-IPN and Universidad Autónoma de Nuevo León
We acknowledge technical help to Yolanda Rodfriguez with HPLC quantification.

CHARACTERIZATION OF A MAIZE KERNEL PROTEIN ASSOCIATED WITH RESISTANCE AGAINST *Aspergillus flavus* INFECTION/AFLATOXIN PRODUCTION

Zhi-Yuan Chen[1], Robert L. Brown[2], Kenneth E. Damann[1] and Thomas E. Cleveland[2]. [1]Department Plant Pathology and Crop Physiology, Louisiana State University Agricultural Center, Baton Rouge, LA; [2]USDA-ARS, Southern Regional Research Center, New Orleans, LA

Aflatoxins are carcinogens produced by *Aspergillus flavus* and *A. parasiticus* during infection of susceptible crops such as corn. Though resistant corn genotypes have been identified, the incorporation of resistance into commercial lines has been slow due to the lack of selectable markers. Through comparisons of kernel embryo proteins of resistant and susceptible genotypes separated using 2-D gels, over a dozen of protein spots were found to be unique or upregulated in resistant embryos, and were sequenced using ESI-MS/MS. One of them is a glyoxalase I (GLXI) based on homology. Its gene was cloned based on amino acid sequences from an embryo cDNA library, which showed high sequence homology to a rice GLXI. Its enzymatic activity was measured in several resistant and susceptible lines, and found to be generally higher in the resistant corn lines. Upon fungal infection, GLXI activity increased in resistant genotypes, whereas it decreased in susceptible ones. The changes in GLXI activity showed significant negative correlation with aflatoxin production in those kernels. The possible function of this glyoxalase in stress and host resistance will also be discussed.

VOLATILES FROM LEAVES OF PISTACHIO SPECIES

James N. Roitman and Gloria B. Merrill. USDA-ARS, Plant Mycotoxin Research, Western Regional Research Center, Albany, CA

Pistachios grow in temperate arid regions of the world, including nations bordering the Mediterranean Sea, Iran (the largest producer), and the U.S, which has now become the world's second largest producer. The genus *Pistacia* is a member of the Anacardaceae (other members include cashew, mango, poison oak and poison ivy) and comprises nine species, only one of which, *Pistacia vera*, is grown commercially in the U.S. for nuts, principally in the central valley of California. The U.S. production of pistachios (last year nearly 250 million pounds) has been steadily increasing over the last 20 years. Insect damage to tree nuts (almonds, walnuts and pistachios) provides openings that allow fungal spores to enter, including those of aflatoxigenic strains of *Aspergillus flavus* and *A. parasiticus*. Because a large percentage all U.S. tree nut crops is exported, reducing aflatoxin levels to meet stringent EU regulations is imperative.

Serious insect pests of nut trees, such as codling moth, navel orangeworm and peach twig borer employ their ability to detect specific volatile organic compounds and mixtures to find potential mates, food sources and locate egg-laying sites. Thoroughly understanding which of the many volatiles emitted by leaves of a particular species attract or repel specific insects would allow control strategies to be developed.

We have so far examined headspace volatile constituents of fresh leaves of *Pistachia vera* and two other pistachio species, *P. chinensis* and *P. lentiscus*. Leaves (800 g) from branches harvested from local orchards were placed in a glass vessel within a few hours of being harvested. The vessel was swept for 18 hr. with 1 L/min of clean air that exited through a trap packed with polymeric absorbent. Extraction of the trap with freshly distilled ether and concentration of the resulting solution 100-1000 fold by distillation afforded a solution that was analyzed by GCMS. The volatiles from all three species comprised mainly monoterpene and sesquiterpene hydrocarbons ($C_{10}H_{16}$ and $C_{15}H_{24}$ respectively). Scarcely any other type of common plant volatile, such as aldehydes, esters, terpene or sesquiterpene alcohols or ethers, was observed, even in trace amounts. However there were marked differences between the three species. The monoterpene, limonene, was the most abundant volatile constituent of the leaves of *P. vera* and *P. lentiscus*, amounting to 46% of the total volatiles of the former and 57% of the latter, but less than 0.2% of the *P. chinensis* volatiles. The monoterpene *trans*-"-ocimene accounted for 33% of the *P. vera* and 20% of the *P. chinensis* volatiles, but amounted to only 1.4% of the volatiles of *P. lentiscus*. The most abundant *P. chinensis* volatile, *cis*-"-ocimene, amounted to nearly 70% of the total but accounted for only 0.2% and 0.8% of the volatiles of *P. lentiscus* and *P. vera* respectively. The 11 sesquiterpene hydrocarbons detected in *P. vera* volatiles were in very low amounts (0.004-0.038%), about 10 fold less than in the volatiles of *P.chinensis* and *P. lentiscus*. Preliminary data on whole pistachio fruit shows that the ratio of sesquiterpene to terpene hydrocarbons increases during the growing season as the fruit matures. The diversity in the headspace volatiles of these three pistachio species offers an opportunity for increasing desirable (insect repelling) volatiles and reducing volatiles that that attract insects by the techniques of genetic manipulation or conventional plant breeding.

GENETIC ANALYSIS OF INHIBITORY PROTEINS FROM MAIZE SEEDS

Kirsten Nielsen, Rebecca S. Boston and Gary A. Payne. Departments of Plant Pathology and Botany, North Carolina State University, Raleigh, NC

The focus of this research project is to identify maize seed proteins that are inhibitory to growth and aflatoxin production by *Aspergillus flavus*. We have identified two proteins with strong antifungal activity. One is a chitinase present in inbred Tex6 that inhibits growth of the fungus by 50% at a concentration of 20µg/mL Our data suggests that chitinase activity in Tex 6 kernels makes a major contribution to the antifungal activity in this corn variety. Partial peptide sequence of the chitinase showed it to be different from previously reported chitinases. We are in the process of cloning the gene that encodes this chitinase from Tex6. The other protein that we have been investigating is the maize endosperm ribosome-inactivating protein, RIP. RIP is a potent translational inhibitor with a putative role in plant defense. RIP was tested for antifungal activity against *A. flavus* and *A. nidulans* with a microculture assay in which fungal conidia treated with RIP or control proteins were monitored over time. Growth of the maize pathogen *A. flavus* was inhibited by RIP. However, the organism could overcome this inhibition by producing a new hyphal tip that led to a branched phenotype. When conidia from a closely related non-pathogenic species, *A. nidulans*, were treated with RIP, we observed a striking decrease in hyphal proliferation, followed by lysis. The lysis and branching phenotypes both occurred at the transition from pre-divisional to post-divisional growth. To determine whether the presence of RIP is important for fungal infection in the field, ears segregating for the opaque-2 mutation (decrease levels of RIP) were inoculated with an *A. flavus* strain expressing the green fluorescent protein and individual kernels were examined for fluorescence. Mutant kernels had higher levels of fluorescence than normal kernels. These results suggest that the antifungal activity of maize RIP plays a role in defense of the kernel against fungal invasion.

USING GFP *Aspergillus flavus* STRAINS TO ASSESS AFLATOXIN RESISTANCE OF PEANUT UNDER DROUGHT

Keith Ingram[1], Corley Holbrook[2], Arthur Wiessinger[3] and Dave Wilson[4].
[1]University of Georgia, Crop and Soil Sciences, Griffin, GA; [2]USDA-ARS, Tifton, GA; [3]Crop Science, North Carolina State University, Raleigh, NC; [4] Plant Pathology, University of Georgia, Tifton, GA

Initial efforts to observe infection of peanut (*Arachis hypogea* L.) roots and pods *in situ* using a minirhizotron camera with a UV light source to observe GFP *Aspergillus flavus* were highly promising. Using the strain of GFP *A. flavus* developed by Jeffery Carey, we observed that inoculum placed on the minirhizotron tube fluoresced brightly. Fluorescence of GFP *A. flavus* revealed decayed roots that were not visible under white light. Fluorescence suggested that GFP *A. flavus* colonized peanut pods. From these experiments we concluded that GFP *A. flavus* offered a new tool to screen peanut germplasm for aflatoxin resistance, to quantify *A. flavus* populations *in situ* from the amount they fluoresced. We hypothesized that *A. flavus* populations may be enhanced by root and pod exudates, and that variation in exudation may be related to aflatoxin resistance. Subsequent research found that fluorescence observed on roots, pods, and pegs decreased with time and that less than 5% of roots and pods observed with a minirhizotron fluoresced and less that 1% of pods or seeds cultured after harvest showed colonization by GFP *A. flavus*.

Our objectives for 2001 were: 1) to develop methods to maximize infection of peanut with *A. flavus*; 2) to develop methods to use GFP *A. flavus* to screen germplasm; and 3) to use GFP *A. flavus* to observe infection pathways in peanut.

Pod cuvette experiment: We grew Georgia Green peanut in 20-L containers in a growth chamber and the Georgia Envirotron in Griffin, GA. About 14 days after plants first flowered, we attached clear acrylic cuvettes (20 cm wide 10 cm high 1.5 cm thick) filled with soil. We applied cracked corn medium inoculated with the strain of GFP *A. flavus* developed by Gary Payne to the soil surface. We imposed different levels of water deficit and observed pegs and pods with a UV-fluorescence dissecting microscope at NCSU. Our observations were: 1) inoculum fluoresced brightly; 2) above ground parts of pegs had lenticel-like structures with fluorescence; 3) fluorescing mycelia were present on some pegs; 4) the system allowed us to observe pods and mycelia on the pod surface *in situ* through the cuvette walls under both white and UV light, but fluorescence was minimal. In summary, pegs and pods grew readily into the cuvettes and we observed mycelia and fluorescence on pegs and pods, but overall, *A. flavus* populations appeared small and levels of fluorescence were low. Experiment on maximizing *A. flavus* infection

We again grew Georgia Green peanut in 20-L containers with four pod cuvettes attached to each container. Plants were grown in growth chambers with relatively high temperatures and with moderate to severe levels of water deficit. We tested inoculation methods in various combinations, all using including both GFP *A. flavus* strains. Inoculation methods for the different cuvettes of each container were 1) spores suspended in water and mixed in soil of cuvettes; 2) cracked corn inoculum applied to the surface of soil in cuvettes; 3) cracked corn inoculum mixed in soil of cuvettes; and 4) no inoculum.

For half of the inoculated containers we sprayed spores suspended in water on plant shoots at 14 and 28 days after first flowering. We also grew four containers of uninoculated plants in a separate growth as absolute controls. About 14 days after spraying the spore suspension on the flowers, we sampled flowers and pegs and observed them with a UV illuminated microscope. Although we observed fluorescing *A. flavus* on the surface of peanut flowers, we did not observe fluorescence in either excised ovules or in pegs excised before entering soil.

QuaCos: Many images of peanut pods illuminated with UV had weak or no apparent fluorescence. In order to quantify amount of fluorescence and to try to detect fluorescence in images having weak fluorescence, we developed software, QuaCos, to analyze the red-green-blue color values of pixels in digital images. QuaCos detected and quantified fluorescence that was otherwise not visible. Initial results show that we can detect variation in fluorescence among genotypes by this method and that there appears to be a relationship between *A. flavus* as estimated from fluorescence of pods *in situ* observed with a minirhizotron on one hand, and subsequent *A. flavus* contamination of pods and seeds cultured after harvest on the other.

Aspergillus EST DATABASES PROVIDE INSIGHTS INTO AFLATOXIN BIOSYNTHESIS RESEARCH.

Perng-Kuang Chang[1], Jiujiang Yu[1], Deepak Bhatnagar[1], Peter Cotty[1], Nancy Keller[2], Rodolfo Aramayo[3], Bruce Roe[4], Doris Kupfer[4], *Aspergillus oryzae* EST Consortium[5] and Thomas Cleveland[1]. [1]USDA-ARS, Southern Regional Research Center, New Orleans, LA; [2]University of Wisconsin, Madison, WI; [3]Texas A&M University, College Station, TX; [4]The University of Oklahoma, Norman, OK. [5]National Institute of Advanced Industrial Science and Technology, Ibaraki, Japan

The use of expressed sequence tags (EST), short "single pass" sequencing reads taken from either the 3' or the 5' end, or both ends of cDNA clones, has become an established approach to identify genes and to analyze profiles of gene expression. ESTs from *Aspergillus flavus*, *A. oryzae* and *A. nidulans*, representing about 10%, 50% and 40%, respectively, coverage of the total genes, are publicly available. These EST databases, though incomplete, nonetheless provide a wealth of information that complements current efforts in aflatoxin research. Several findings were derived from the analysis of these *Aspergillus* EST databases. First, genes involved in aflatoxin and sterigmatocystin production are differentially expressed in the early stage of growth of *A. flavus* and *A. nidulans*. Second, the transcripts derived from aflatoxin and sterigmatocystin gene clusters represent about 1.5 % of the population of total transcripts. Third, two genes, *norA* and *aflJ*, located in the *A. flavus* and *A. parasiticus* aflatoxin gene clusters, were also found in the *A. oryzae* EST database. This result suggests that these two genes are probably under different regulation, since the homolog of the aflatoxin pathway regulatory gene, *aflR*, is not expressed in *A. oryzae*. Fourth, the homologs of *omtA* and *ordA* were not found in the *A. nidulans* EST database, which suggests that the accumulation of sterigmatocystin as the end product in *A. nidulans* is due to lack of these genes or gene expression. Aflatoxin and sterigmatocystin gene expression profiles obtained from the whole genome approach would allow us to devise better strategies to eliminate toxin contamination of agricultural commodities.

PANEL DISCUSSION: Potential Use of Natural Products for Prevention of Fungal Invasion and / or Aflatoxin Biosynthesis in Crops

Panel Chair: Russell Molyneux

Panel Members: Perng-Kuang Chang, Z.-Y Chen, Doralinda Guzman-de-Pena, James Roitman

Summary of Presentations: The moderator for this session was Bob Klein of the California Pistachio Commission. The recent EU regulation of 2 ppb aflatoxin B_1 (4 ppb total aflatoxins) is a major concern for the treenut industry because a significant proportion of the total production of almonds, pistachios and walnuts is exported. There were six presentations including presentations on natural constituents of walnut, endogenous enzymes and other proteins in maize, plant growth regulators, and volatile hydrocarbons emitted by pistachio leaves that act as aflatoxin resistance factors, inhibit toxin biosynthesis, or have antifungal properties. In all cases, the precise mechanisms are unclear. Another presentation in this session summarized the use of a green fluorescence protein-containing strain of *Aspergillus flavus* to screen peanut germplasm for aflatoxin resistence. The final presentation highlighted the use of existing *Aspergillus* expression sequence tag databases as a readily available resource for gleaning useful information about the control of aflatoxin biosynthesis.

Summary of Panel Discussion: Several questions were directed to Keith Ingram about the methods used for quantification of the green fluorescence protein using image analysis software (QuaCos). It was pointed out that the QuaCos software could be adjusted for background fluorescence but that it is easiest to stick with the green fluorescence for which the software has been optimized. With regards to the longest interval after inoculation that fluorescence can be detected, it was pointed out that while 4-5 weeks was possible, the length might be extended beyond this period with manipulation of the Quacos software. The green fluorescence protein construct has never been put on the isolated ovule. Questions also arose about the ability of other auxins, besides naphthalene acetic acid, to control the growth of toxigenic *Aspergillus* species. Apparently, all other auxins tested were found to negative. With regards to access to EST databases, it was pointed out that all the databases are available on the internet and that interested individuals should contact P.K. Chang for the URLs.

INHIBITION OF AFLATOXIN SYNTHESIS IN CORN GRAIN BY NAPHTHALENE ACETIC ACID

Gloria Godoy [1], Carlos M. Bucio-Villalobos[2] and Doralinda Guzmán-de-Peña[3]. [1]Instituto Tecnológico de Celaya, Guanajuato; [2] Instituto de Ciencias Agrícolas, Universidad de Guanajuato; [3]Laboratorio de Micotoxinas, Unidad Irapuato. Centro de Investigación y Estudios Avanzados. IPN. México

Previous studies with the plant growth regulator, naphthalene acetic acid (NAA), on the synthesis of aflatoxin by *Aspergillus parasiticus* have shown an inhibitory effect in synthetic minimal media. Even though the mechanism of this inhibition has not been elucidated it was considered important to analyze the behavior of NAA when applied directly to corn grain. Since "if an aflatoxin inhibitor is to be of potential value in a food crop, it should be active in the field and during storage". Two types of experiments were performed. First, experiment "A" using sterile corn grain with and without inoculation of *A. parasiticus* and 50 mM of NAA. In these experiments spore count and aflatoxin quantification were performed after 6 days of incubation at 28^0 C. Second, experiment "B" using corn naturally contaminated with fungi and aflatoxin was used and NAA was added as powder, at 0, 112mg and 300mg / 50 g of dry corn. After 6 days of incubation at 28^0 C, changes in fungal population were evaluated as incidence in 100 corn kernels. Extraction of aflatoxin was done by the modified CB-1 method and HPLC quantification. The experiments were performed twice with 5 replicates in each treatment. The results of experiment "A" showed that naphthalene acetic acid (50 mM) inhibited aflatoxin synthesis in corn grain with statistical significance. The results of experiment "B" showed that the high concentration of dry NAA causes changes in fungal populations and decreased aflatoxin content in naturally contaminated corn.

This project was partially supported by CONACYT-México.
We acknowledge technical help to Yolanda Rodriguez for HPLC quantification.

CHARACTERIZATION OF OXYGENASES INVOLVED IN THE *Aspergillus*/SEED INTERACTION

D. Tsitsigiannis[1], Terri M. Kowieski[1], R. Plattner[2] and N. Keller[1].
[1]Department of Plant Pathology, University of Wisconsin, Madison, WI;
[2]USDA-ARS, Northern Center for Agricultural Utilization Research, Peoria, IL

A promising strategy to control aflatoxin contamination of plants is to utilize knowledge gained from molecular studies of the effect of plant gene products on *Aspergillus* growth and aflatoxin (AF)/sterigmatocystin (ST) production, and modify levels of these products accordingly. Sexual, asexual and AF/ST development in *Aspergillus* species is affected by both seed and fungal derivatives of linoleic acid (LA). Seed defense enzymes, lipoxygenases (*lox*) convert LA to 13S-hydroperoxylinoleic acid, 13S-HPODE (inhibits AF/ST production) and/or 9S-HPODE (promotes AF/ST gene transcription). Both compounds stimulate *Aspergillus* asexual development, possibly by mimicking affects of psi factors (precocious sexual inducers); endogenous *Aspergillus* sporogenic factors derived from LA. The major objectives of this study are a) to clone peanut seed *lox* genes, b) to clone the psi producing gene(s) in *A. nidulans* and c) to characterize the role of these genes in *Aspergillus* pathogenesis and subsequent AF/ST production. Fragments of putative novel *pnlox* genes have been subcloned from *Aspergillus* infected and non-infected peanut seeds. Characterization of these peanut *lox* genes will reveal the LA derived products (9S-HPODE, 13S-HPODE or both) and could represent the first molecular markers with potential to identify genotypes with enhanced resistance or susceptibility to AF contamination. In *A. nidulans* three putative *ppo* (psi producing oxygenases) genes have been cloned. Deletion of *ppoA* in *A. nidulans* decreases conidial and ST production. Further studies using prototrophs of the Δ*ppoA* strain will clarify the role of this gene in asexual, sexual and ST production.

THE ROLE OF DELTA 9 STEARIC ACID DESATURASE IN *Aspergillus* DEVELOPMENT

Richard Wilson[1], Ron Plattner[2] and Nancy Keller[1]. [1]Department of Plant Pathology, University of Wisconsin, Madison, WI; [2]USDA, ARS, Northern Center for Agricultural Utilization Research, Peoria, IL

We have cloned and sequenced a delta9-stearic acid desaturase encoding gene from *Aspergillus nidulans*. *Aspergillus* spp. are opportunistic pathogens of oil seed crops that secrete the highly carcinogenic mycotoxin aflatoxin (AF). *A. nidulans* is a model laboratory organism that produces the related toxic compound, sterigmatocystin (ST). Interestingly, a number of fatty acid molecules derived from both seed and *Aspergillus* can effect sporulation and ST/AF production of these fungi. *Aspergillus* sporogenic molecules, termed psi factors, are synthesized from oleic, linoleic and linolenic fatty acids. A mutant of the delta 12-oleic acid desaturase gene (*odeA*) is defective in the synthesis of linoleic acid from oleic acid. It produces large amounts of oleic acid, but only trace amounts of linoleic acid, has altered sporulation and abherrant ST and psi factor production. We wanted to further study the effects of fatty acid metabolism on *Aspergillus* development, ST/AF production and seed colonization by preventing the conversion of stearic acid to oleic acid, creating a mutant with presumably no psi factors. An EST-clone with high delta 9-stearic acid desaturase identity was found in the *Aspergillus nidulans* EST-library database. We used this clone to design a plasmid vector that would replace the chromosomal delta 9-stearic acid desaturase gene, termed *sdeA*, with the *pyrG* gene. The resulting *sdeA* disruption strain grows poorly at all temperatures, and is especially retarded in growth at 22 ^{o}C. At 37 ^{o}C, asexual conidiation is reduced compared to the wild type, and sexual spore production was not observed. In addition, the delta *sdeA* mutant produces many hulle cells, sterile cells involved in sexual development, whereas the wild type produces no detectable hulle cells under the same growth conditions. Interestingly, TLC analysis of mycotoxin production shows it has elevated amounts of ST relative to the wild type. Therefore, the disruption of the *A. nidulans sdeA* gene, encoding a delta 9-stearic acid desaturase, leads to reduced asexual and sexual spore development, increased hulle cell production, and a marked increase in ST production. One major goal for the future will be the elucidation of the mechanism by which ST production is increased in strains carrying the disrupted *sdeA* gene and determine if this mechanism is conserved for AF production in *A. parasiticus* and *A. flavus*. The discovery of gene mutations that lead to the up-regulation of ST/AF production could be important tools for understanding AF/ST biosynthesis and be future targets for mycotoxin control.

ANALYSIS OF HEADSPACE VOLATILES FROM PISTACHIO LEAVES

Gloria B. Merrill and James N. Roitman. USDA-ARS, Plant Mycotoxin Research, Western Regional Research Center, Albany, CA

We have examined the volatile organic compounds emitted by fresh leaves of three pistachio species: *Pistacia chinensis, P. lentiscus* and *P. vera*. *Pistacia chinensis* is a shapely ornamental tree and is grown for that purpose. *P. lentiscus,* is an evergreen shrubby tree grown for its oleoresin, used in perfumes chewing gum, varnishes, pharmaceuticals and dental adhesives; its shoots find use adding pleasant aromas to springtime floral bouquets and its berries are used to flavor sweets and liqueurs. *P. vera* is the species grown commercially in the U.S. for production of pistachio nuts. The U.S. production of pistachio nuts (last year nearly 250 million pounds) has been steadily increasing over the last 20 years.

Insect damage to tree nuts (almonds, walnuts and pistachios) provides openings that allow fungal spores, including those of aflatoxigenic strains of *Aspergillus flavus* and *A. parasiticus* to enter. Because a large portion all U.S. tree nut crops is exported, reducing aflatoxin levels to meet stringent EU regulations is imperative. Insect pests of nut trees, such as codling moth, navel orangeworm and peach twig borer employ their ability to detect specific volatile organic compounds and mixtures to find potential mates, food sources and locate egg-laying sites. Thoroughly understanding which of the many volatiles emitted by leaves of a particular species attract or repel specific insects would allow control strategies to be developed.

Purified air was passed over the fresh leaves and exited through an absorbent trap. Ether rinsing of the trap followed by concentration by distillation afforded an ethereal solution of volatiles that was analyzed by GCMS. The volatiles from all three species comprised mainly monoterpene and sesquiterpene hydrocarbons ($C_{10}H_{16}$ and $C_{15}H_{24}$ respectively). Other types of common plant volatile substances, such as aldehydes, ketones, esters, terpene or sesquiterpene alcohols or ethers, were noticeably absent, except for traces of a few. However there were distinct differences between the three species. The monoterpene, limonene, was the most abundant volatile constituent of the leaves of *P. vera* and *P. lentiscus*, amounting to 46% of the total volatiles of the former and 57% of the latter, but less than 0.2% of the *P. chinensis* volatiles. The monoterpene *trans*-"-ocimene accounted for 33% of the *P. vera* and 20% of the *P. chinensis* volatiles, but amounted to only 1.4% of the volatiles of *P. lentiscus*. The most abundant *P. chinensis* volatile, *cis*-"-ocimene, amounted to nearly 70% of the total but accounted for only 0.2% and 0.8% of the *P. lentiscus* and *P. vera* volatiles respectively. The 11 sesquiterpene hydrocarbons detected in *P. vera* volatiles were in very low amounts (0.004-0.038%), about 10 fold less than in *P.chinensis* and *P. lentiscus*. Our preliminary data on whole pistachio fruit headspace volatiles show that the ratio of sesquiterpene to terpene hydrocarbons increases during the growing season as the fruit matures. The diversity in the headspace volatile profiles of these three pistachio species offers an opportunity for increasing desirable (insect repelling) volatiles and reducing volatiles that that attract insects by the techniques of genetic manipulation or conventional plant breeding.

REDUCTION OF AFLATOXIN PRODUCTION BY AROMA COMPOUNDS

Sui-Sheng T. Hua, Nancy Han and Henry Shu. USDA-ARS, Western Regional Research Center, Albany, CA

Aflatoxin B_1 is highly toxic to the liver of mammals and the most potent carcinogen known. Infection of almonds, pistachios and other tree nuts by *Aspergillus flavus* before harvest and during storage results in the biosynthesis and accumulation of aflatoxin B_1. This mycotoxin poses a significant problem in food quality and food safety worldwide. The Food and Drug Administration has set a maximum level of 20 ppm of aflatoxin in food intended for human consumption. Recently the Commission of the European Community has set a more restrictive level of 2 ppm for aflatoxin B_1. Because the application of toxic fungicides to prevent the growth of *A. flavus* on food are unacceptable approaches thus searching for natural compounds inhibitory to fungal growth and aflatoxin biosynthesis may have potential application. The effects of six aroma compounds on the growth of *A. flavus* and on the reduction of aflatoxin biosynthesis are being investigated. Fungal spores were suspended in Tween-80 water (0.05%). The number of spores in the suspension was determined using Beckman Coulter Multisizer II. Suspensions of spores were prepared to 10^5 spores / ml. Spores (5 l) were inoculated in the center of a PDA plate containing the compounds at concentrations ranging from 0 to 500 ppm in triplicate. The plates were incubated at 28 C in the dark. The growth of the fungus was determined by measuring the diameter of the colony on the agar plate. The inhibitory effects of the compounds on spore germination and hyphae elongation were monitored by viewing with a microscope. Aflatoxin was extracted from the fungal mat and agar by methanol and analyzed by high performance liquid chromatography (HPLC) on a Hewlett Packard model 1050 Chemstation. Aflatoxin B_1 was detected by a fluorescent detector with excitation at 365 nm and emission at 455 nm. The inhibitory activities of 300 ppm of eugenol, methyl jasmonate, 6-amyl-"-pyrone, *-decanolactone, compound x and compound y on the growth of *A. flavus* 18-3 and 827 were determined Compound x was the most effective one which inhibited the fungal growth completely after 9 days of incubation. Compound y, eugenol and 6-amyl-a-pyrone were also effective in inhibiting the fugal growth, the sizes of the fungal colonies were only 10-30% of the control. The same pattern of inhibition was observed in strain of *A. flavus*, AF 2-01. The six compounds were also tested on two strains of *Aspergillus nigel* and one strain of *Aspergillus ochraceus*. Compound y is the most active in inhibiting the germination and growth of *A. nigel*. No fungal growth was observed after 11 days of incubation. Again compounds x and y showed good activity in inhibiting the growth of *A. ochraceus*. Aflatoxin biosynthesis of *A. flavus* was inhibited by 57 to 100% depending on the concentrations of compound y and *-decanolactone. Even though there was fungal growth in the presence of *-decanolactone but the compound apparently exerted inhibitory action to aflatoxin production. The effects of eugenol, compound x, methyl jasmonate and 6-amyl-"--pyrone on aflatoxin biosynthesis of *A. flavus* are in progress.

PROGRESS IN DEVELOPMENT OF THE PEAR ESTER KAIROMONE TO CONTROL CODLING MOTH AND *Aspergillus* IN WALNUTS

D.M. Light[1], A.L. Knight[2], S. Lingren[3], C.A. Henrick[3] and B. Lingren[3].
[1]USDA-ARS, Western Regional Research Center, Plant Mycotoxin Research Unit, Albany, CA; [2]USDA-ARS, YARL, Wapato, WA; [3]Trece, Inc., Salinas, CA

The chief avenue of *Aspergillus* invasion of tree nuts is through insect damage by primarily moth larvae. Our contribution to the integrated mission to eliminate aflatoxin is targeted at diminishing and managing insect-caused nut damage through the development and use of novel, species-specific control systems based on host-plant semiochemicals. Because adult female moths lay eggs that develop into damaging larvae, monitoring or controlling female codling moth adults would create an opportunity for more precise population prediction and control efficacy.

We hypothesized that the preferred host of codling moth, pear, may contain kairomonal volatiles attractive to adult females. Headspace odor trapping and GC-MS analysis led to the identification and screening of numerous (*ca*. 100) pear volatiles in California walnut orchards. Through these efforts a single compound, ethyl (2*E*, 4*Z*)-2,4-decadienoate (Et-*E,Z*-DD), was identified as a kairomone that captures both male and female codling moth adults in baited sticky traps. Through a cooperative research and development agreement, and a recently approved patent, between USDA/ARS and Trécé, Inc., a global research program was initiated in 1999 to define the possible commercial uses for the compound.

Ethyl (2*E*, 4*Z*)-2,4-decadienoate is a non-toxic, naturally occurring fruit volatile. Investigations using gas chromatographic-flame ionization detector coupled with an electroantennographic detector (GC-EAD) revealed that Et-*E,Z*-DD is the most stimulating compound for codling moths of 16 major volatiles identified from pear. Field evaluations further demonstrated that Et-*E,Z*-DD is as attractive to male and female codling moths as codling moth pheromone is to male codling moths. Et-*E,Z*-DD is attractive to both virgin and mated codling moths. Furthermore, Capturing other, non-target insects is extremely rare at these load rates, suggesting that Et-*E,Z*-DD is also as species-specific as pheromone.

For females, this attraction was demonstrated to be, in part, a host finding adaptation, with females ovipositing a greater proportion of their eggs near Et-*E,Z*-DD impregnated septa. In addition to attracting codling moth adults, Et-*E,Z*-DD was found to be attractive to codling moth neonate larvae. The unique set of attributes of Et-*E,Z*-DD in attracting codling moths presents multiple opportunities for development of novel IPM products. The attraction of both female and male and virgin and mated moths monitoring advantages, including more reliable treatment thresholds, more reliable treatment timing, and ability to monitor in mating disruption orchards.

The kairomone, Et-*E,Z*-DD, presents several exciting opportunities for moth control, either alone or in combination with pheromone. Et-*E,Z*-DD attracts both males and females when presented alone and in combination with pheromone. An attracticide formulated with Et-*E,Z*-DD should be much more effective than that formulated with pheromone alone. Trécé, Inc. has made significant progress in developing slow-release

formulations for such a use. Finally, there exists and opportunity to improve disruption tactics with the use or addition of Et-*E,Z*-DD. Inundation of orchards with this compound could potentially improve pheromonal mating disruption by increase false trail following by male codling moths, disrupt oviposition by females, or confuse host finding by larvae.

PARTICIPANTS

Abbas, Hamed K
USDA-ARS, CG & PRU
141 Experiment Station Rd.
Stoneville, MS 38776
Tel: (662) 686-5313
Fax: (662) 686-5218
Email: habbas@ars.usda.gov

Antilla, Larry C
Arizona Cotton Research
& Protection Council
3721 E. Wier Ave.
Phoenix, AZ 85040
Tel: (602) 438-0059
Fax: (602) 438-0407
Email: lantilla@azcotton.com

Bacon, Charles
USDA-ARS, Russell Research Center
Toxicology & Mycotoxin Research Unit
PO Box 5677
Athens, GA 30604-5677
Tel: (706) 546-3158
Fax: 706-546-3116
Email: cbacon@saa.ars.usda.gov

Baker, James L
USDA-ARS
Western Regional Research Center
Plant Mycotoxin Research Unit
800 Buchanan St.
Albany, CA 94710-1105
Tel: (510) 559-5855
Fax: (510) 559-5777
Email: jlbaker@pw.usda.gov

Bennett, Joan
Tulane University
Department of Cell and Molecular
Biology
New Orleans, LA 70118
Tel: (504) 862-8101
Fax: (504) 865-6785
Email: jbennett@tulane.edu

Bennett, Greg
Center for Human Molecular Genetics
Dept. of Cell Biology & Anatomy
Munroe-Meyer Institute
985455 Nebraska Medical Center
Omaha, NE 68198-5455
Tel: (402) 559-6715

Betschart, Antoinette A
USDA-ARS
Pacific West Area
800 Buchanan St.
Albany, CA 94710-1105
Tel: (510) 559-6060
Fax: (510) 559-5779
Email: abetschart@pw.ars.usda.gov

Bhatnagar, Deepak
USDA-ARS
Southern Regional Research Center
1100 Robert E. Lee Blvd.
New Orleans, LA 70124
Tel: (504) 286-4388
Fax: (504) 286-4419
Email: dbhatnag@srrc.ars.usda.gov

Brooks, Thomas
USDA-ARS
Corn Host Plant Resistance
P.O. Box 5367
Mississippi State, MS 39762

Brown, Robert
USDA-ARS
Southern Regional Research Center
1100 Robert E. Lee Blvd.
New Orleans, LA 70124
Tel: (504) 286-4359
Fax: (504) 286-4419
Email: rbrown@srrc.ars.usda.gov

Brown, Daren
USDA-ARS, NCAUR
Mycotoxin Research Unit
1815 N. University St.
Peoria, IL 61604- 3999
Tel: (309) 681-6230
Fax: (309) 681-6689
Email: browndw@ncaur.usda.gov

Bucio-Villalobos, Carlos M.
Universidad de Guanajuato
Manuel Mendivil 337 Fracc. CD. Deportiva
Irapuato, GTO 36612
Mexico
Tel: (4) 6244118
Fax: (4) 6244118
Email: bucioc@dulcinea.ugto.mx

Campbell, Bruce C
USDA-ARS, WRRC
Pacific West Area
800 Buchanan St.
Albany, CA 94710-1105
Tel: (510) 559-5846
Fax: (510) 559-5737
Email: bcc@pw.usda.gov

Cao, Yangguo
University of Georgia
Coastal Plain Experiment Station
Tifton, GA 31793
Tel: (229) 387-2334
Fax: (229) 387-2321
Email: ycao@tifton.cpes.peachnet.edu

Cary, Jeff
USDA-ARS
Southern Regional Research Center
1100 Robert E. Lee Blvd.
New Orleans, LA 70124
Tel: (504) 286-4264
Fax: (504) 286-4419
Email: jcary@srrc.ars.usda.gov

Chang, Perng-Kuang
USDA-ARS
Southern Regional Research Center
1100 Robert E. Lee Blvd.
New Orleans, LA 70124
Tel: (504) 286-4208
Fax: (504) 286-4419
Email: pkchang@srrc.ars.usda.gov

Chen, Zhi-Yuan
Louisiana State University
Baton Rouge, LA 70124
Tel: (504) 286-4345
Fax: (504) 286-4419
Email: zchen@nola.srrc.usda.gov

Chlan, Caryl A
Univ. of Louisiana - Lafayette
Biology Dept.
P.O. Box 42451
Lafayette, LA 70504
Tel: (337) 482-5916
Fax: (337) 482-5660
Email: cchlan@louisiana.edu

Cleveland, Thomas
USDA-ARS
Southern Regional Research Center
1100 Robert E. Lee Blvd.
New Orleans, LA 70003
Tel: (504) 286-4387
Fax: (504) 286-4269
Email: eclevela@srrc.ars.usda.gov

Cotty, Peter J.
USDA-ARS
Southern Regional Research Center
1100 Robert E. Lee Blvd.
New Orleans, LA 70124
Tel: (504) 286-4391
Fax: (504) 286-4496
Email: pjcotty@srrc.ars.usda.gov

Damann, Ken
L.S.U. Agriculture Center
302 Life Sciences Bldg
Baton Rouge, LA 70803
Tel: (225) 388-1401
Fax: (225) 578-1415
Email: kdamann@agctr.lsu.edu

Dandekar, Abhaya
University of California
Department of Pomology
1059 Wickson Hall
One Shield Ave
Davis, CA 95616
Tel: (530) 752-7784
Fax: (530) 752-8502
Email: amdandekar@ucdavis.edu

Desjardin, Anne
USDA-ARS, NCAUR
Mycotoxin Research Unit
1815 N. University St.
Peoria, IL 61604- 3999
Tel: (309) 681-6378
Fax: (309) 681-6689
Email: desjarae@ncaur.usda.gov

Donaldson, Gerald
Texas Corn Producers
3127 Donaldson Road
Wharton, TX 77488
Tel: (979) 282-9592
Fax: (979) 282-9760
Email: donfarm@wcnet.net

Dorner, Joe W
USDA-ARS
National Peanut Lab
P.O. Box 509
Dawson, GA 31742
Tel: (912) 995-7408
Fax: (912) 995-7416
Email: jdorner@nprt.usda.gov

Doster, Mark
University of California
Kearney Agricultural Center
9240 South Riverbend Ave.
Parlier, CA 93648
Tel: (559) 646-6585
Fax: (559) 646-6562
Email: mark@uckac.edu

Dowd, Patrick F.
USDA-ARS
NCAUR, BAR
1815 N. University St.
Peoria, IL 61604-3999
Tel: (309) 681-6242
Fax: (309) 681-6686
Email: dowdpf@mail.ncaur.usda.gov

Dowell, Floyd
USDA-ARS, Grain Marketing
& Production Research Center
Engineering Research Unit
1515 College Avenue
Manhattan, KS 66502
Tel: (785) 776-2753
Fax: (785) 776-2792
Email: fdowell@usgmrl.ksu.edu

Du, Liangheng
University of Nebraska
Department of Chemistry
Room 647, Hamilton Hall
Lincoln, NE 68588
Tel: (402) 472-2998
Fax: (402) 472-9402
Email: ldu@unlserve.unl.edu

Duran, Norma L.
USDA-ARS
US Water Conservation Laboratory
4331 E. Broadway Rd.
Phoenix, AZ 85040
Tel: (602) 437-1702 ext. 258
Fax: (602) 437-5291
Email: NDuran@uswcl.ars.ag.gov

Ehrlich, Kenneth
USDA-ARS
Southern Regional Research Center
P.O. Box 19687
New Orleans, LA 70179
Tel: (504) 286-4369
Fax: (504) 286-4419
Email: ehrlich@srrc.ars.usda.gov

Ellis, Leland
USDA-ARS, NPS
RM 4-2192
5601 Sunnyside Ave
Beltsville, MD 20705-5139
Tel: (301) 504-4788
Fax: (301) 504-4725
Email: lce@ars.usda.gov

Eversole, Kelly
Eversole Associates
3208 Park View Road
Chevy Chase, MD 20815
Tel: (301) 951-3345
Fax: (301) 951-1846
Email: kellye@eversolassociates.com

Gelineau-van Waes, Janée
Center for Human Molecular Genetics
Dept. of Cell Biology & Anatomy
Munroe-Meyer Institute
985455 Nebraska Medical Center
Omaha, NE 68198-5455
Tel: (402) 559-6715
Fax: (402) 559-4001
Email: jvanwaes@unmc.edu

Glenn, Anthony
USDA-ARS, Russell Research Center
Toxicology & Mycotoxin Research Unit
Athens, GA 30604-5677
Tel: (706) 546-3119
Fax: (706) 546-3116
Email: aglenn@saa.ars.usda.gov

Gradziel, Thomas
University of California
Department of Pomology
1047 Wickson Hall
One Shield Ave
Davis, CA 95616
Tel: (530) 752-1575
Fax: (530) 752-8502
Email: tmgradziel@ucdavis.edu

Gray, Gary D
Blue Diamond Almonds
1802 C. Street
Sacramento, CA 95812
Tel: (916) 446-8384
Fax: (916) 446-8697
Email: ggray@bdgrowers.com

Griffith, Tom
ABERCO, Inc.
9430 Lanham-Severn Road
Seabrook, MD 20706
Tel: (301) 459-7090
Fax: (301) 577-9041
Email: taberco@aol.com

Guo, Baozhu
USDA-ARS, CPMRU
P.O. Box 748
Tifton, GA 31793
Tel: (229) 387-2326
Fax: (229) 387-2321
Email: bguo@tifton.cpes.peachnet.edu

Guzman-de-Pena, Doralinda
Laboratorio de Micotoxinas
Unidad Irapuato
Centro de Investigacion y Estudios
Avanzados
Km p.6 Ira-Leon Apto 629
Irapuato, GTO 36690
Mexico
Tel: (4) 6239600
Email: dguzman@ira.cinvestav.mx

Hammond, Bruce
Monsanto Company
800 North Lindberg Blvd
St. Louis, MO 63167
Tel: (314) 694-8482
Fax: (314) 694-8575
Email:
bruce.g.Hammond@monsanto.com

Hawkins, Leigh
USDA-ARS
Corn Host Plant Resistance
P.O. Box 5367
Mississippi State, MS 39762

Holbrook, Corley
USDA-ARS
Plant Pathology Research
P.O. Box 748
Tifton, GA 31793
Tel: (229) 386-3176
Fax: (229) 391-3701
Email:
Holbrook@tifton.cpes.peachnet.edu

Horn, Bruce W
USDA-ARS
National Peanut Research Lab
P.O. Box 509
1011 Forrester DR, SE
Dawson, GA 31742-0509
Tel. (229) 995-7410
Fax: (229) 995-7416
Email: bhorn@nprl.usda.gov

Hua, Sui-Sheng
USDA-ARS, WRRC
Pacific West Area
800 Buchanan St.
Albany, CA 94710-1105
Tel: (510) 559-5905
Fax: (510) 559-5777
Email: ssth@pw.usda.gov

Hurley, J. Michael
DFA of California
1855 S. Van Ness
Fresno, CA 93721
Tel: (559) 233-0604
Fax: (559) 266-2199
Email: mikehurtey@dfaofca.com

Ingram, Keith
University of Georgia
1109 Experiment Rd
Griffin, GA 30223-1797
Tel: (770) 412-4045
Fax: (770) 229-3215
Email: kingram@griffin.peachnet.edu

Isakeit, Thomas
Texas A & M University
2132 TAMU
College Station, TX 77843-2132
Tel: (979) 862-1340
Fax: (979) 845-6483
Email: t-isakeit@tamu.edu

Jacobs, Merle
Almond Board of California
1150 9th St.
Suite 1500
Modesto, CA 95354
Tel: (209) 343-3222
Fax: (209) 549-8267
Email: mjacobs@almondboard.com

Keller, Henry
Keller Grain Inc
1150 US Hwy 90 E
Castroville, TX 78009
Tel: 830 931-2579
Fax: 830 538-2668
hmkeller@stic.net

Kendra, David
Syngenta Seeds, Inc.
317 330th St.
Stanton, MN 55018-4308
Tel: (507) 663-7636
Fax: (507) 645-7519
Email: dave.kendra@syngenta.com

King, Edgar
USDA-ARS
CG & PRU
141 Experiment Station Rd.
Stoneville, MS 38776
Tel: (662) 686-5266
Fax: (662) 686-5265
Email: eking@ars.usda.gov

Kistler, H. Corby
USDA-ARS
Cereal Disease Laboratory
1551 Lindig Street
St. Paul, MN 55108
Tel: (612) 625-9774
Fax: (651) 649-5054
Email: hckist@puccini.cdl.umn.edu

Klein, Robert E
California Pistachio Commission
1318 East Shaw Ave.
Suite 420,
Fresno, CA 93710
Tel: (559) 221-8294
Fax: (559) 221-8044
Email: bobk@pistachios.org

Light, Douglas
USDA-ARS
Pacific West Area
800 Buchanan St.
Albany, CA 94710-1105
Tel: (510) 559-5831
Fax: (510) 559-5777
Email: dlight@pw.usda.gov

Maddox, Joyce
Center for Human Molecular Genetics
Dept. of Cell Biology & Anatomy
Munroe-Meyer Institute
985455 Nebraska Medical Center
Omaha, NE 68198-5455
Tel: (402) 559-2863
Email: jmaddox@unmc.edu

Mahoney, Noreen E
USDA-ARS
Pacific West Area
800 Buchanan St.
Albany, CA 94710-1105
Tel: (510) 559-5971
Fax: (510) 559-5777
Email:nmahoney@pw.ars.usda.gov

Maragos, Chris
USDA-ARS
NCAUR, BAR
1815 N. University Street
Peoria, IL 61604
Tel: (309) 681-6266
Fax: (309) 681-6267
Email: maragocm@ncaur.usda.gov

McAlpin, Cesaria
USDA-ARS
NCAUR, BAR
1815 N. University Street
Peoria, IL 61604-3999
Tel: (309) 681-6248
Fax: (309) 681-6686
Email: mcalpice@ncaur.usda.gov

Merrill, Gloria
USDA-ARS, WRRC
Pacific West Area
800 Buchanan Street
Albany, CA 94710-1105
Tel: (510) 559-5809
Fax: (510) 559-5779
Email: gbm@pw.usda.gov

Michailides, Themis J
University of California
Kearney Agricultural Center
9240 South Riverbend Ave.
Parlier, CA 93648
Tel: (559) 646-6546
Fax: (559) 646-6593
Email: themis@uckac.edu

Molyneux, Russell
USDA-ARS
Pacific West Area
800 Buchanan St.
Albany, CA 94710-1105
Tel: (510) 559-5812
Fax: (510) 559-5777
Email: molyneux@pw.ars.usda.gov

Moore, Steven H
Dean Lee Research Station
8105 East Campus Avenue
Alexandria, LA 71302
Tel: (318) 473-6520
Fax: (318) 473-6535
Email: smoorcalex@aol.com

Muhitch, Michael
USDA-ARS
NCAUR, BAR
1815 N. University Street
Peoria, IL 61604-3999
Tel: (309) 681-6368
Fax: (309) 681-6686
Email: muhitchm@mail.ncaur.usda.gov

Murphy, Emory M
Georgia Peanut Commission
P. 0. Box 967
Tifton, GA 31793
Tel: (229) 386-3475
Fax: (229) 386-3501
Email: emory@gapeanuts.com

Nierman, William
TIGR-The Institute for Genomic
Research
9712 Medical Center Drive
Rockville, MD 20850
Tel: (301) 838-0200
Fax: (301) 838-0208
Email: wneirman@tigr.org

Nigh, Edward
Arizona Ag Con Inc

O'Callaghan, John
Microbiology Department
University College Cork
College Road
Cork, Ireland
Tel: 353 21 4902952
Fax: 353 21 4903101
Email: j.ocallaghan@ucc.ie

Odvody, Gary
Texas A&M Research & Extn Ctr
Rt. 2, Box 589
Corpus Christi, TX 78406-9704
Tel: (361) 265-9201
Fax: (361) 265-9434
Email: g-odvody@tamu.edu

Ozias-Akins, Peggy
University of Georgia - Tifton Campus
P.O. Box 748
Tifton, GA 31793-0748
Tel: (912) 386-3902
Fax: (912) 386-3356
Email: ozias@tifton.cpes.peachnet.edu

Park, Douglas
Division of Natural Products (HFS-345)
Center for Food Safety
and Applied Nutrition
200 C Street, SW
Washington, DC 20204
Tel: (202) 260-2694
Fax: (202) 205-4422
Email: dpark@cfsan.fda.gov

Payne, Gary
Department of Plant Pathology
Box 7616
North Carolina State University
Raleigh, NC 27695-7616
Tel: (919) 515-6994
Fax: (919) 515-7716
Email: gary_payne@ncsu.edu

Perkin, James
Monsanto Company
8350 Minnegan Road
Waterman, IL 60556
Tel: (815) 758-9524
Fax: (815) 758-3117
Email: jim.perkins@monsanto.com

Plattner, Ronald
USDA-ARS
NCAUR, BAR
1815 N. University St.
Peoria, IL 61604
Tel: (309) 681-6241
Fax: (309) 681-6689
Email: plattnrd@ncaur.usda.gov

Procter, Robert
USDA-ARS
NCAUR, BAR
1815 N. University St.
Peoria, IL 61604
Tel: (309) 681-6381
Fax: (309) 681-6689
Email: proctorh@ncaur.usda.gov

Pryor, Barry
University of Arizona
Department of Plant Pathology, 204 Forbes
P.O. Box 210036
Tucson, AZ 85721
Tel: (520) 626-5312
Fax: (520) 621-9290
Email: bmpryor@ag.arizona.edu

Puntase, Janjira
University of Georgia
1109 Experiment Rd
Griffin, GA 30224
Tel: (770) 412-4703
Email: janjira@gaes.griffin.peachnet.edu

Rajasekaran, Kanniah
USDA-ARS
Southern Regional Research Center
1100 Robert E. Lee Blvd.
New Orleans, LA 70124-4305
Tel: (504) 286-4482
Fax: (504) 286-4217
Email: krajah@srrc.ars.usda.gov

Ramos, David
Walnut Marketing Board
Department of Pomology
1035 Wickson Hall
University of California
One Shield Ave
Davis, CA 95616
Tel: (530) 752-0508
Email: deramos@ucdavis.edu

Riley, Ronald
USDA-ARS, Russell Research Center
Toxicology & Mycotoxin Research Unit
950 College Station Rd
Athens, GA 30604-5677
Tel: (706) 546-3377
Fax: (706) 546-3116
Email: rriley@saa.ars.usda.gov

Robens, Jane F
USDA-ARS
National Program Staff
5601 Sunnyside Avenue
Bldg. 4, Rm. 2184
Beltsville, MD 20705-5138
Tel: (301) 504-5381
Fax: (301) 504-5467
Email: jfr@ars.usda.gov

Rocheford, Torbert R
University of Illinois
Department of Crop Sciences
S-116 Turner Hall
MC-046
1102 S Goodwin Ave.
Urbana, IL 61801
Tel: (217) 333-9643
Fax: (217) 333-9817
Email: trochefo@uiuc.edu

Roitman, James
USDA-ARS, WRRC
Pacific West Area
800 Buchanan St.
Albany, CA 94710-1105
Tel: (510) 559-5784
Fax: (510) 559-5777
Email: jnr@pw.usda.gov

Schatzki, Thomas
USDA-ARS
Pacific West Area
800 Buchanan St.
Albany, CA 94710-1105
Tel: (510) 559-5672
Fax: (510) 559-5777
Email: tom@pw.usda.gov

Seiber, James N.
USDA-ARS
Pacific West Area
800 Buchanan St.
Albany, CA 94710-1105
Tel: (510) 559-5600
Fax: (510) 559-5963
Email: jseiber@pw.usda.gov

Showker, Jency
USDA-ARS, Russell Research Center
Toxicology & Mycotoxin Research Unit
950 College Station Rd
Athens, GA 30604-5677
Tel: (706) 546-3377
Fax: (706) 546-3116
Email: jshowker@saa.ars.usda.gov

Simmons, Kay
USDA-ARS, NPS
RM 4-2210
5601 Sunnyside Ave
Beltsville, MD 20705-5139
Tel: (301) 504-5560
Fax: (301) 504-6191
Email: kws@ars.usda.gov

Snyder, Jennifer
Corn Refiners Association, Inc.
1701 Pennsylvania, Ave. NW
Ste 950
Washington, DC 20006
Tel: (202) 331-1634
Fax: (202) 331-2054
Email: jsynder@corn.org

Sobotev, Victor S
USDA-ARS
National Peanut Research Lab
1011 Forrester DR, SE
Dawson, GA 31742-0509
Tel: (229) 995-7446
Fax: (229) 995-7416
Email. vsobolev@nprl.usda.gov

Tsitsigiannis, Dimitrios
Department of Plant Pathology
University of Wisconsin
1640 Linden Dr
Madison, WI 53706
Tel: (608) 265-2060
Fax: (608) 263-2626
Email: dimtsi@plantpath.wisc.edu

Valentine, Howard E
The American Peanut Council
10625 Big Canoe
Jasper, GA 30143
Tel: (706) 579-1755
Fax: (706) 579-1754
Email: pnuttech@alltel.net

Wakelyn, Phillip J
National Cotton Council
1521 New Hampshire Avenue, NW
Washington, DC 20036
Tel: (202) 745-7805
Fax: (202) 483-4040
Email: pwakelyn@cotton.org

Walker, Scott
Route 3 Box 331C
Monsanto
Haubstadt, IN 47639
Tel: 812 768-5676
Fax: 812 768-6586
scott.l.walker@stl.monsanto.com

Weissinger, Arthur K
North Carolina State University
P.O. Box 7620
Raleigh, NC 27695-7620
Tel. (919) 515-2704
Fax: (919) 515-7959
Email: arthur@unity.ncsu.edu

White, Don
Department of Crop Science
N425 Turner Hall
1102 South Godwin Ave.
University of Illinois
Urbana, IL 61801
Tel: (217) 333-1093
Fax: (217) 244-1230
Email: d-white@uiuc.edu

Wicklow, Donald T
USDA-ARS
NCAUR, BAR
1815 N. University St.
Peoria, IL 61604-3999
Tel: (309) 681-6243
Fax: (309) 681-6686
Email: wicklodt@mail.ncaur.usda.gov

Widstrom, Neil
USDA-ARS
Crop Genetics and Breeding Research Unit
P.O. Box 748
Tifton, GA 31793
Tel: (229) 387-2341
Fax: (229) 387-2321
Email:
nwidsto@tifton.cpes.peachnet.edu

Williams, William
USDA-ARS
Corn Host Plant Resistance Research
810 Highway 12 East
Mississippi State, MS 39762-5367
Tel: (662) 325-2735
Fax: (662) 325-8441
Email: pwilliams@dorman.msstate.edu

Wilson, Richard
Department of Plant Pathology
University of Wisconsin
1640 Linden Dr
Madison, WI 53706
Tel: (608) 265-2060
Fax: (608) 263-2626
Email: rwilson@plantpath.wisc.edu

Windham, Gary
USDA-ARS
Corn Host Plant Resistance Research
P.O. Box 5367
Mississippi State, MS 39762
Tel: (662) 320-7402
Fax: (662) 325-8441
Email: gwindham@ra.msstate.edu

Woloshuk, Charles
Purdue University
Dept. of Botany and Plant Pathology
1155 Lilly Hall of Life Sciences
West Lafayette, IN 47907-1155
Tel: (765) 494-3450
Fax: (765) 494-0363
Email: woloshuk@btny.purdue.edu

Youngker, Chuck K
Youngker & Youngker 11
P.O. Box 158
Buckeye, AZ 85326
Tel: (602) 386-2565

Yu, Jiujiang
USDA-ARS
Southern Regional Research Center
1100 Robert E. Lee Blvd.
New Orleans, LA 70124
Tel: (504) 286-4405
Fax: (504) 286-4419
Email: jiuyu@srrc.ars.usda.gov

Zorner, Paul
Demegen Inc.
1051 Brinton Rd
Pittsburgh, PA 15221
Tel: (760) 533-9435
Fax: (412) 241-2161
Email: paul@telanetix.com

1st Fungal Genomics,
2nd Fumonisin Elimination
and
14th Aflatoxin Elimination Workshops
October 23-26 2001
Phoenix, Arizona